

Non-Toxic HYDRANAL® Karl Fischer Reagents



Titration

- Non-Toxic Karl Fischer Reagents
- Water Determination in Plastics

Standards

- Biodiesel Calibration Standards

Chromatography

- Ion Pair Reagents for HPLC
- Mobile Phase Additives for LC-MS
- Resins & Media
- Gel and Capillary Electrophoresis

Sensorics

- Selectophore® Ionophores

Analytical Microbiology

- Identification of *Listeria monocytogenes*

New Product Corner

- KOH and HCl Isopropanol Solutions



Small Unit Packaging Service Thorough documentation gives accurate, uninterrupted paper trail to meet the most rigorous quality requirements



Picture Don Hobbs, Director of Marketing, Supelco

Dear Colleague,

You know Sigma-Aldrich supplies innovative products to the analytical chemistry market, but did you know we offer innovative services as well? In one such service, Small Unit Packaging, we convert large, bulk samples of solids or liquids into convenient smaller units – ampuls, vials, metalized pouches – for our customers. Every aspect of the process, from packaging and testing to labeling and documentation, is defined by the customer to meet their specific criteria and regulatory demands. In this Editorial, we'll take a closer look at one dimension of our Small Unit Package service: the paper trail.

The need for Small Unit Packaging is prevalent in the pharmaceutical industry, where small samples from large containers of a chemical, perhaps an API, bulk drug substance, raw material or excipient batch, are required for various analytical applications across different manufacturing and testing sites. GLP protocols dictate that when the repackaged sample is to be used as an analytical reference standard, it is crucial that the standard is traceable back to the original sample.

An uninterrupted paper trail – beginning with recording receipt of the bulk chemical at our dock and concluding when it is returned repackaged to the customer – is a critical part of our Small Unit Packaging service. Two documents serve as the foundation of the paper trail: the Protocol Sheet and the Narrative Report. At the beginning of the process, we meet with the customer to establish all the details of the project, including the chemical to be repackaged, type of vessel to use, unit size, packaging specification, testing procedures, etc.

With the details agreed upon, both parties sign off on a Protocol Sheet. Once the process is established, a Narrative Report document is generated for each batch of sample we repackage. The Narrative Report summarizes all activities that have taken place with respect to the chemical while it is in our hands. The Narrative Report is completed by the production supervisor and reviewed by our QA manager. Both production and QA must sign off on the Narrative Report document. A copy of the Narrative and every record we generate during the packaging is placed in a notebook and shipped to the customer.

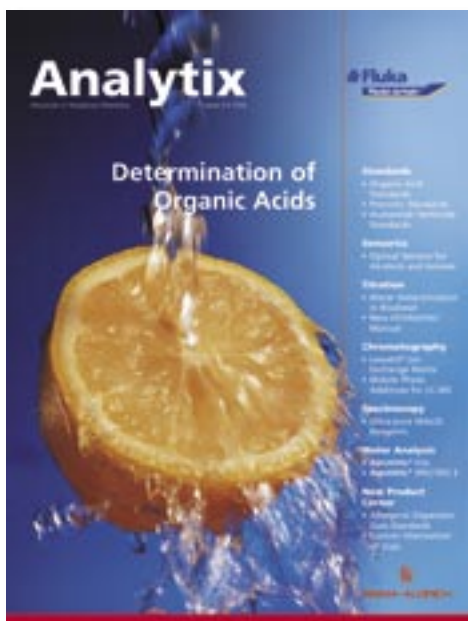
The paper trail of our Small Unit Packaging service gives it accountability and verifies our attention to every detail in the preparation of the samples. Of course there is no charge for this paperwork; it is just a part of the service we provide.

We have many customers who rely on our Small Unit Packaging service because of the convenience, time- and cost-savings it offers them. If you or your company could benefit as well, please give us a call and let Supelco become a valued supply-chain partner.

We look forward to hearing from you!

Regards,

Don Hobbs
Director of Marketing
Supelco/Division of Sigma-Aldrich
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Non-Toxic HYDRANAL® Karl Fischer Reagents Based on Ethanol Improving upon the safety without compromising performance

By Michael Jeitziner, Market Segment Manager Analytical Reagents ... mjeitziner@sial.com
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Introduction

A central focus of the on-going improvements to our HYDRANAL® line of pyridine-free Karl Fischer reagents is the reduction or elimination of toxic reagents. One such reagent is methanol, which is widely used as the solvent in the titration vessel and as a solvent for other K-F reagents. As useful as methanol is, it is also toxic.

Figure 1
Titration of 25 mg water using HYDRANAL®-CompoSolver E versus methanol

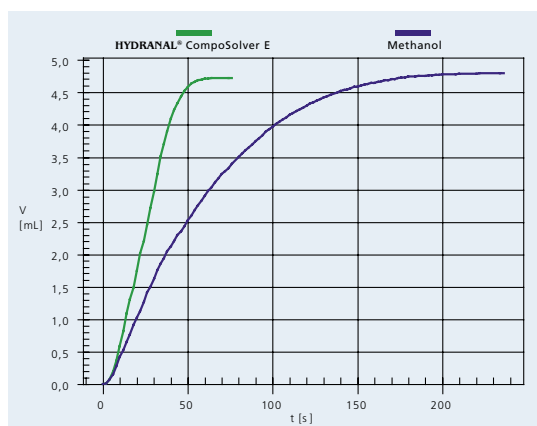


Figure 2
Titration of 5 mL acetone with HYDRANAL®-CompoSolver E versus methanol

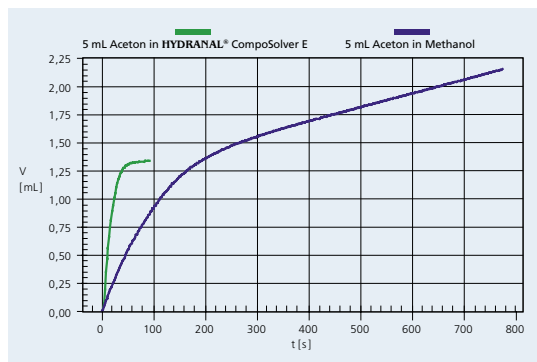


Table 1 Solubility of hydrophobic samples in 30 mL of methanol and various HYDRANAL® solvents

Sample	HYDRANAL®-CompoSolver E	Methanol	HYDRANAL®-Solvent E	HYDRANAL®-Solvent
Benzene	> 30 mL	> 30 mL	> 30 mL	10 mL
Petrol benzene	> 30 mL	22 mL	30 mL	22 mL
n-Hexane	> 30 mL	13 mL	22 mL	7 mL
Cyclohexane	> 30 mL	11 mL	24 mL	6 mL
Iso-Octane	> 30 mL	10 mL	18 mL	4 mL
1-Decene	> 30 mL	6 mL	12 mL	2 mL
1-Tetradecene	25 mL	1 mL	3 mL	< 1 mL
Dodecane	21 mL	< 1 mL	3 mL	< 1 mL

Methanol is classified as poisonous according to chemical regulations in the European Union. Poisonous chemicals represent not only a danger to the user, but are also subject to certain restrictions regarding shipping and storage.

Eliminating Methanol: HYDRANAL® E-Types

Our improvement efforts have led to the successful replacement of methanol with non-toxic ethanol. These ethanol-containing reagents, the HYDRANAL® E-types (patent pending), represent the first non-toxic Karl Fischer reagent line for volumetric and coulometric titration.

Not only can ethanol-based K-F reagents replace methanol-based reagents in nearly all applications, they are actually more suitable for hydrophobic samples and ketones. Titration in HYDRANAL®-CompoSolver E is much faster than in pure methanol (**Figure 1**). Additionally, the end points in ethanol are stable and result in very accurate water content measurements. Moisture content of many ketones, including acetone, can be determined using HYDRANAL®-CompoSolver E with HYDRANAL®-Composite. Alcoholic side-reactions with ketones are often less pronounced in ethanol than in methanol (**Figure 2**). The solubility for long-chained hydrocarbons in ethanol-based HYDRANAL® reagents is improved over methanol and methanol-containing reagents (**Table 1**). HYDRANAL®-CompoSolver E is a particularly effective solubilizer.

Use of HYDRANAL® E-Types

The following procedure is recommended for volumetric titrations with HYDRANAL® E-types in one- and two-component systems (see **Figures 3 and 4**):

1. Fill the burette with HYDRANAL®-Composite or HYDRANAL®-Titrant E
2. Add HYDRANAL®-CompoSolver E or HYDRANAL®-Solvent E into the titration vessel
3. Titrate it to dryness with HYDRANAL®-Composite or HYDRANAL®-Titrant E
4. Add the sample
5. Titrate the water content with HYDRANAL®-Composite or HYDRANAL®-Titrant E

Summary of Features of HYDRANAL® E-Types

- Reduced toxicity over methanol-containing reagents
- Pyridine-free, like all HYDRANAL® reagents
- Used for both volumetric and coulometric K-F titrations



Table 2 Comparison of Methanol- and Ethanol-Based Karl Fischer Reagents

Reagents based on methanol	Reagents based on ethanol (E-types)
Volumetric titration: One-component reagents	
HYDRANAL®-Composite (non-hazardous) (Solvent DEGEE, see ECETOC Technical Report No 17) used with HYDRANAL®-Methanol dry (toxic)	HYDRANAL®-Composite (non-hazardous) (Solvent DEGEE, see ECETOC Technical Report No 17) used with HYDRANAL®-CompoSolver E
Volumetric titration: Two-component reagents	
HYDRANAL®-Solvent (toxic)	HYDRANAL®-Solvent E
HYDRANAL®-Titrant 5 (toxic)	HYDRANAL®-Titrant 5E
HYDRANAL®-Titrant 2 (toxic)	HYDRANAL®-Titrant 2E
Coulometric titration	
HYDRANAL®-Coulomat AD (toxic)	HYDRANAL®-Coulomat E
HYDRANAL®-Coulomat AG (toxic)	

Table 3 Applications where Use of Ethanolic Reagents is Preferred

Laboratory Report No.*	Sample	Results
L539	Surface preservative, wood decking protector	Does not work with ethanol, but works with CompoSolver E
L540	5-Hydroxy-1-methylpyrazole	In methanol weak side reaction, but works fine in CompoSolver E
L456	Peppermint oil and spearmint oil	In methanol weak side reaction, but works fine in CompoSolver E
L452	Lacquer	Works fine for lacquers and dyes

*Laboratory Reports can be obtained by FAX or email by contacting our HYDRANAL® Laboratories (hhoffman@europe.sial.com). You can also find the full list on our website www.sigma-aldrich.com/hydranal

Table 4 Product Listing; HYDRANAL® E-type Reagents

Cat. No.	Brand	Description	Used for	Package Size
34730	Riedel-de Haën	HYDRANAL®-Solvent E	Ethanolic solvent (two component reagent), use with HYDRANAL®-Titrant E	500 mL, 1 L, 2.5 L
34732	Riedel-de Haën	HYDRANAL®-Titrant 5E	Ethanolic titrant (two component reagent)	100 mL, 500 mL, 1 L, 2.5 L
34723	Riedel-de Haën	HYDRANAL®-Titrant 2E	Ethanolic titrant (two component reagent)	1 L
34734	Riedel-de Haën	HYDRANAL®-CompoSolver E	Ethanolic working medium (one component reagent), use with HYDRANAL®-Composite	1 L, 2.5 L
34726	Riedel-de Haën	HYDRANAL®-Coulomat E	Ethanolic anode and cathode reagent for coulometric Karl Fischer titration	500 mL

- Additives increase reaction rate and conductivity of ethanol
- End-point color appears visually more intense compared to methanol
- Replaces most methanolic methods
- Compatible with all titration equipment
- Fulfills the requirements of ISO EN DIN 14001
- Improved solubility for long chained hydrocarbons, especially HYDRANAL®-CompoSolver E
- Ketones, like acetone, can be titrated in HYDRANAL®-CompoSolver E, but not in methanol.
- HYDRANAL®-Coulomat E is a one-component reagent used as both anolyte and catholyte for coulometry

Applications

Table 2 shows the corresponding ethanolic reagents for the methanolic systems. For most applications, there is little or no difference between using methanolic or ethanolic reagents. There are several applications where ethanolic K-F reagents are actually preferred. A few of these are listed in **Table 3**.

See page 7 for contact information.

Figure 3

One-Component System with E-Types

HYDRANAL®-Composite



HYDRANAL®-CompoSolver E

Figure 4

Two-Component System with E-Types

HYDRANAL®-Titrant E



HYDRANAL®-Solvent E

Determination of the Water Content in Polymers by Karl Fischer Titration

Successful analysis is accomplished using a Karl Fischer oven and **HYDRANAL**[®] reagents designed specifically for this application

By Helga Hoffmann, Technical Support **HYDRANAL**[®] Manager ... hhoffman@europe.sial.com



Plastics manufacture is an important global chemical industry. Accurate chemical analysis of raw and intermediate materials is critical to ensure performance, form, function and stability of the final product. The plastic polymer is often supplied in a granule format that is then used to make the finished product. For optimum workability and high quality products, the water content of the granules must not exceed certain process-specific levels. For example, compact disks (CDs) are made from polycarbonate. If the water content of the granules exceeds 0.1%, small bubbles are formed during extrusion, making the resulting CDs unusable. Therefore, the water content must first be determined and, if necessary, the granules have to be pre-dried.

However, moisture determination in polymers can be challenging. The polymer chains can bind water within their structure, sometimes holding it so strongly that it evaporates very slowly. Using a loss on drying technique to measure moisture is time-consuming. Additionally, because other components of the formulation may also be volatile, this technique can lead to falsely high results. The only means to determine the true water content is by Karl Fischer titration.

Table **HYDRANAL**[®] Reagents for Karl Fischer oven titrations

Cat. No.	Description	Package Size
34739	HYDRANAL [®] -Coulomat AG-Oven	500 mL
34836	HYDRANAL [®] -Coulomat AG	500 mL, 1L
34810	HYDRANAL [®] -Coulomat AD	500 mL
34840	HYDRANAL [®] -Coulomat CG	25 mL, 50 mL
34741	HYDRANAL [®] -Methanol dry	1 L, 2.5 L
34748	HYDRANAL [®] -Water Standard KF-Oven	10 g
34828	HYDRANAL [®] -Water Standard 1.00	40 mL
34241	HYDRANAL [®] -Molecular sieve 0.3nm	250 g, 1 kg
34788	HYDRANAL [®] -Humidity Absorber	500 g, 1 kg

Karl Fischer titration of polymer samples

The medium (solvent in the titration vessel) used in a Karl Fischer titration must meet several important criteria:

1. An alcoholic medium is required to fulfill the requirements of a correct K-F equation.
2. The medium has to permit a proper indication of the end point of the titration.
3. Very importantly, the medium must permit total dissolution of the sample or, at the very least, its complete release of moisture.

Alcohol is obviously an important component to a successful Karl Fischer titration. However, most polymers do not dissolve readily in the alcohol-containing media. (An exception is polycarbonate, but this procedure is not suitable for continual use. See **HYDRANAL**[®] Laboratory Report L 129.) Polymers often require the indirect method using a Karl Fischer oven. This technique works by heating the polymer sample to a suitable temperature while a carrier gas (dry air or nitrogen) sweeps the heat-liberated components of the sample into a coulometric cell where only the water is titrated.

The Karl Fischer oven method

Temperature is critical to a successful water determination using the Karl Fischer oven. The temperature must be high enough to ensure rapid and complete release of moisture, but not be so high as to cause decomposition of the polymer or additives. Such decomposition could ultimately cause side reactions that lead to erroneous results.

It is good practice when using the Karl Fischer oven method to test each sample at several different temperatures. Automated equipment is available that permits the operator to program and run temperature ramps, starting at 50 °C and gradually heating the polymer to the final temperature at the programmed rate.

Examples of polymers titrated using the Karl Fischer oven

Polycarbonate polymer is the first example presented here. Titration of a polycarbonate sample using a Karl Fischer oven from 50 – 250 °C is shown in **Figure 1**, where the blue trace is the water released during heating and the red trace is the rate of release. Two observations can be made. First, up to 200 °C the

Figure 1 Temperature ramp 50 – 250 °C on polycarbonate sample

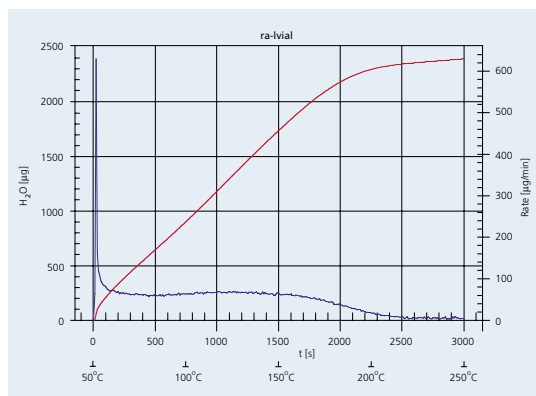
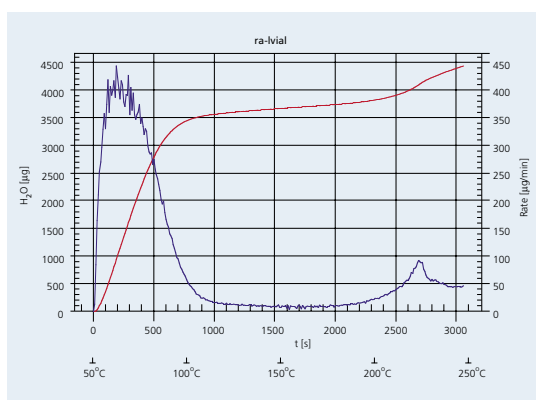


Figure 2 Temperature ramp 50 – 250 °C on plastic film sample



sample releases water. Second, the product is stable to at least up to 250 °C. We recommend an evaporation temperature between 160 °C and 200 °C as described in Laboratory Report L 127.

The second example is a plastic film made of polyamide/ethylene vinylalcohol. The temperature ramp in **Figure 2** clearly shows that an evaporation temperature between 120 °C and 150 °C is ideal. At 180 °C the material begins to decompose. Further details of this analysis can be found in Laboratory Report L 526.

Karl Fischer oven procedure for polymer samples

- 5 mL **HYDRANAL**[®]-Coulomat CG is placed in the cathode compartment of the coulometry cell. Approximately 100 mL **HYDRANAL**[®]-Coulomat AG-Oven is added to the same fill level. The cell without a diaphragm only requires **HYDRANAL**[®]-Coulomat AG-Oven.
- The machine is switched on and automatically dry titrates. If the drift is below 10 µg/min. and is stable, the sample is heated.

HYDRANAL[®]-Coulomat AG-Oven can also be replaced with **HYDRANAL**[®]-Coulomat AD or **HYDRANAL**[®]-Coulomat AG.

Achieve accurate analyses using **HYDRANAL**[®] reagents

Using reagents and accessories designed specifically for the Karl Fischer oven technique and some practical guidelines will maximize the success of the analysis. The flow of carrier gas will volatilize some of the methanol in the reagent, reducing its volume in the anodic compartment. At the end of each working day, it should be replaced by addition of **HYDRANAL**[®]-Methanol dry. The best analyte is **HYDRANAL**[®]-Coulomat AG oven. Because of its formulation, the amount of volatilized methanol is reduced giving a more stable drift. The ideal drying agent for the carrier gas is **HYDRANAL**[®]-Molecular sieve 0.3 nm or **HYDRANAL**[®]-Humidity Absorber. The reliability of a titration method can be tested using the certified **HYDRANAL**[®]-Water Standard KF-Oven. The reliability of the coulometric vessel can be checked with the certified **HYDRANAL**[®]-Water standard 1.00.

List of **HYDRANAL**[®] Laboratory Reports

Plastic film	L 526
Polyamide	L 126
Polyamide 66	L 167, L 174
Polycarbonate	L 127, L 129
Polyethylene	L 128, L 193
Polypropylene	L 181, L 194
Polyurethane	L 124, L 303
Polymers with a KF Oven	L 328



For more information on these or any of the **HYDRANAL**[®] products or applications or to request a Laboratory Report, please contact us at:

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Biodiesel Calibration Standards for ASTM D6584 Multi-component kits simplify analyses and save preparation time

By Vicki Yearick, Environmental Market Segment Manager ... vyearick@sial.com



Low levels of free and bound glycerin are critical to the performance of biodiesel fuels. Free glycerin and bound glycerin both contribute to the formation of deposits on injector nozzles, pistons, valves, filters and storage tanks. ASTM D6584 provides a standardized method for the determination of free glycerin and total (free + bound) glycerin. Commercial biodiesel producers use this method to determine if a production run of B100 (100% biodiesel methyl esters) can be sold or needs to be reworked.

The method outlined in ASTM D6584 provides for the quantitative determination of free and total glycerin in B100 by high temperature gas chromatography. The analytes are silylated using N-methyl-N-(trimethylsilyl)tri fluoroacetamide (MSTFA). Five multi-component calibration solutions containing glycerin, mono-, di-,

and triglycerides must be prepared and then derivatized for the analysis.

Sigma Aldrich, through its Supelco brand, offers individual multi-component solutions specifically designed for use with ASTM D6584. These pre-made solutions save the analyst valuable preparation time. As an added convenience, all five solutions plus an internal solution are available in kit form. Each biodiesel standard comes with a Certificate of Composition and instructions for sample derivatization using MSTFA derivatization reagent (Cat. No. 394866) also available from Sigma-Aldrich.

For further reading on biodiesel analysis:

[1] Analytix, Issue 4, 2005 (available at www.sigma-aldrich.com/analytix)

Table 1 Biodiesel Standard Solutions from Sigma-Aldrich; Kits and Internal Standard Solutions*

Cat. No.	Description	Package Size
44899-U	ASTM D6584 Standard Solution 1: glycerin (5 µg/mL), monoolein (100 µg/mL), diolein (50 µg/mL), triolein (50 µg/mL) in pyridine	1 x 1 mL
44914-U	ASTM D6584 Standard Solution 2: glycerin (15 µg/mL), monoolein (250 µg/mL), diolein (100 µg/mL), triolein (100 µg/mL) in pyridine	1 x 1 mL
44915-U	ASTM D6584 Standard Solution 3: glycerin (25 µg/mL), monoolein (500 µg/mL), diolein (200 µg/mL), triolein (200 µg/mL) in pyridine	1 x 1 mL
44916-U	ASTM D6584 Standard Solution 4: glycerin (35 µg/mL), monoolein (750 µg/mL), diolein (350 µg/mL), triolein (350 µg/mL) in pyridine	1 x 1 mL
44917-U	ASTM D6584 Standard Solution 5: glycerin (50 µg/mL), monoolein (1000 µg/mL), diolein (500 µg/mL), triolein (500 µg/mL) in pyridine	1 x 1 mL
44898-U	ASTM D6584 Standard solution Kit without Internal Standards, varied, pyridine (Kit contains 1 each: 44899-U, 44914-U, 44915-U, 44916-U and 44917-U)	5 x 1 mL
44896-U	Butanetriol (CAS# 42890-76-6) Internal Standard #1, 1000 µg/mL in pyridine	1 x 5 mL
44897-U	Tricaprin (CAS# 621-71-6) Internal Standard #2, 8000 µg/mL in pyridine	1 x 5 mL

* Other biodiesel compound standards are available. Please call or visit our web site: www.sigma-aldrich.com. Use our Advanced Search Engine and type "biodiesel" in the Keyword field.

Mobile Phase Additives for LC-MS. Part 3: The Neutral Salts This is the third article in a five part series on mobile phase additives for LC-MS to appear in each issue of Analytix in 2006

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Although organic acids are the most common mobile phase additive for HPLC separations that employ MS detection, it may be necessary under certain circumstances to use more neutral conditions, either because the analytes are sensitive to acids or do not exhibit optimal resolution at low pH. When acids are not suitable, volatile salts, like ammonium formate or ammonium acetate, may be the additives of choice (Table 1). However, compared to organic acids their use is much more complex. One issue is the limited solubility of the salts in organic solvents; another issue is the changing pH value during a gradient. On the other hand, the mildly acidic pH provided by the salts permits both positive and negative ion mode detection.

This short article will discuss the characteristics, benefits and practical use of the ammonium salts of acetic and formic acid as LC-MS mobile phase additives. All analytical conditions and test compounds were the same as described in part 1 of this series [1], except the concentration of raffinose, which was 100 ng/mL in this study. Additionally, a four peptide mixture of bradykinin analogues was used in one experiment. The salts were dissolved in the aqueous part of the mobile phase at a concentration of 0.1% w/v. The organic part of the mobile phase was used either without any additive or as ready-to-use LC-MS CHROMASOLV® blends also containing 0.1% w/v additive (Table 2).



Table 1 List of Sigma-Aldrich LC-MS additives

Cat. No.	Brand	Description*	Package Size	Packaging
40967	Fluka	Trifluoroacetic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
40967	Fluka	Trifluoroacetic acid, puriss p.a., eluent additive for LC-MS	10 x 1 mL	Glass ampules
56302	Fluka	Formic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
49199	Fluka	Acetic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
49916	Fluka	Propionic acid, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle
55674	Fluka	Ammonium formate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
49638	Fluka	Ammonium acetate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
61333	Fluka	Sodium citrate tribasic dihydrate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
40867	Fluka	Ammonium bicarbonate, puriss p.a., eluent additive for LC-MS	50 g	HDPE bottle
44273	Fluka	Ammonium hydroxide solution 25%, puriss p.a., eluent additive for LC-MS	100 mL	HDPE bottle
65897	Fluka	Triethylamine, puriss p.a., eluent additive for LC-MS	50 mL	HDPE bottle

* "puriss" quality grade is defined as >98.5% assay, <0.1% ash, and specification n + 0.001, d + 0.001 with no extraneous color and a homogeneous appearance. "p.a." or pro analysi denotes a product with guaranteed trace impurity levels and/or suitability for the indicated analytical application.

Table 2 Selection of LC-MS CHROMASOLV® blends

Cat. No.	Brand	Description	Package Size	Packaging
34674	Riedel-de Haën	Water with 0.1% ammonium acetate LC-MS CHROMASOLV®	2.5 L	amber bottle
34670	Riedel-de Haën	Methanol with 0.1% ammonium acetate LC-MS CHROMASOLV®	2.5 L	amber bottle
34669	Riedel-de Haën	Acetonitrile with 0.1% ammonium acetate LC-MS CHROMASOLV®	2.5 L	amber bottle
34668	Riedel-de Haën	Acetonitrile with 0.1% formic acid LC-MS CHROMASOLV®	2.5 L	amber bottle

The main issue when using ammonium acetate or ammonium formate as additives is their solubility, which is very good in water, sufficient in methanol to obtain a concentration of 0.1% w/v, but not in acetonitrile. This is a problem since acetonitrile is the organic solvent of choice for most separations. The effect is shown in **Figure 1**. When using pure acetonitrile as the organic part in gradient elution against 0.1% ammonium acetate in water, the apparent pH will rise and influence the separation, worsening it in most cases (curve A). The same is true when running a gradient with methanol containing 0.1% ammonium acetate in both solvents (curve B). To address the solubility issue, Sigma-Aldrich has developed a special blend (Cat. No. 34669, patent pending), which contains 0.1% w/v of ammonium acetate in acetonitrile stabilized with acid. This acid-stabilization has three desirable effects: the salt is kept in solution, the blend is stable against decomposition and the system is buffered. It also keeps the pH in the mildly acid range when using both the aqueous and organic components as buffered blends (curve C) and when using the acetonitrile blend not as intended, but with pure water as the aqueous solvent (curve D).

Figure 2 EIC (positive ion mode) of test compounds with ammonium acetate as additive in both aqueous and organic components (conditions C in Figure 1)

Red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine, and dark green is propazine.

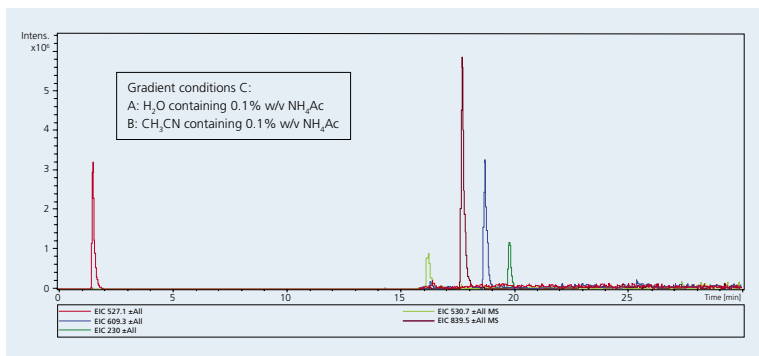
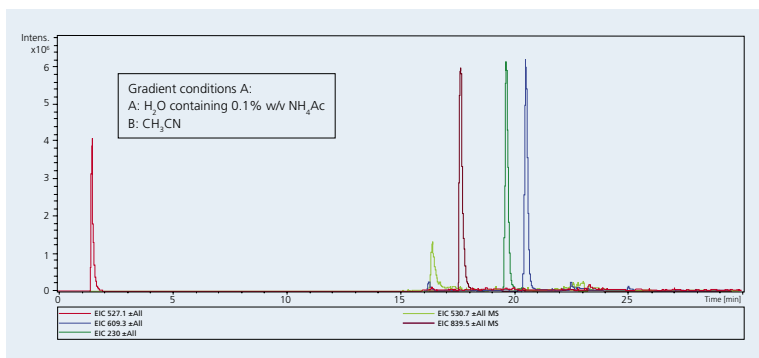


Figure 3 EIC (positive ion mode) of test compounds with ammonium acetate in the aqueous component only (conditions A in Figure 1)

Red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine, and dark green is propazine.



Besides affecting the apparent pH, using buffered mobile phase components has a significant impact on the separation and ionization of the test compounds in this study. Under conditions C (**Figure 2**) and conditions A (**Figure 3**), reserpine (blue peak) is shifted in retention time, and both reserpine and propazine (dark green) exhibit different degrees of ionization. The effect is even more pronounced on the four bradykinin analogues (**Figure 4**). Resolution was greatest using the buffered conditions C (upper trace). However, unfortunately it also had a higher tendency to form sodium adducts when using ion trap instruments compared to triple quads [2].

Similar observations are made for ammonium formate. **Table 3** lists the changes in pH when using 0.1%

Figure 1 Apparent pH curves using buffered and unbuffered blends

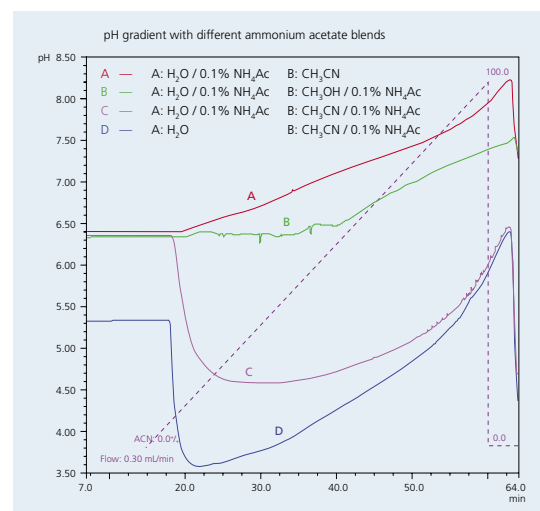


Figure 4 EIC (positive ion mode) of bradykinin analogues with both components containing buffered ammonium acetate (upper trace) and with 0.1% ammonium acetate / acetonitrile, not buffered (lower) (conditions C and A in Figure 1)

1 = bradykinin 1-6, 2 = Lys-Ala³-bradykinin, 3 = bradykinin, 4 = des-Arg¹-bradykinin, 5 = impurity

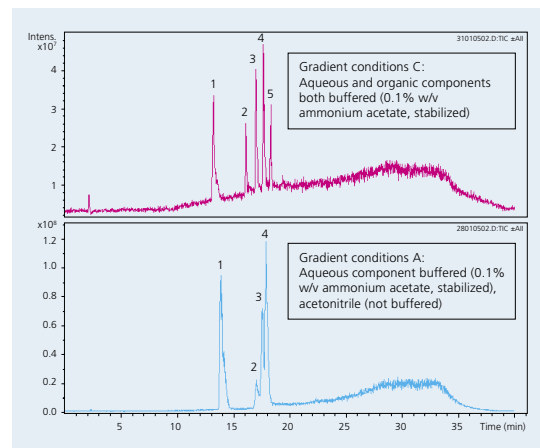


Figure 5 EIC (positive ion mode) of test compounds with ammonium formate in the aqueous component only

Red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine and dark green is propazine.

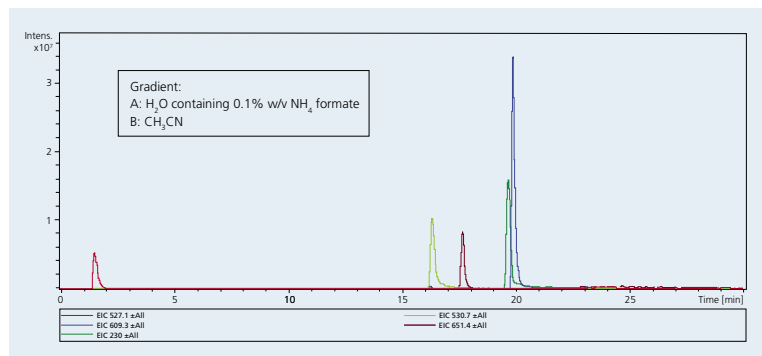


Figure 6 EIC (negative ion mode) of test compounds with ammonium formate in the aqueous component only Red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine; propazine is not detected in neg. ion mode.

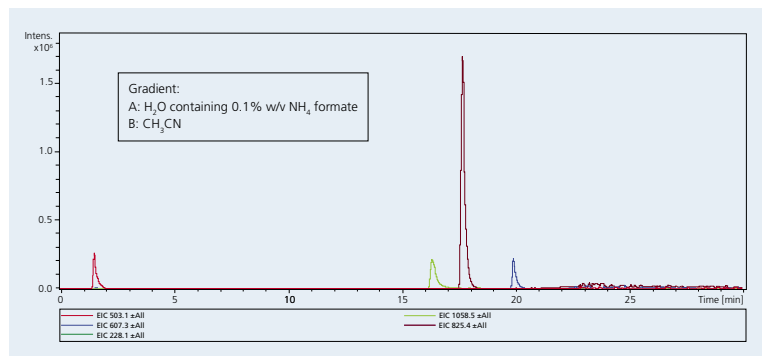


Figure 7 EIC (positive ion mode) of test compounds with ammonium formate in the aqueous component and formic acid in the organic component

Red trace is raffinose, green is bradykinin, dark red is digoxin, blue is reserpine and dark green is propazine.

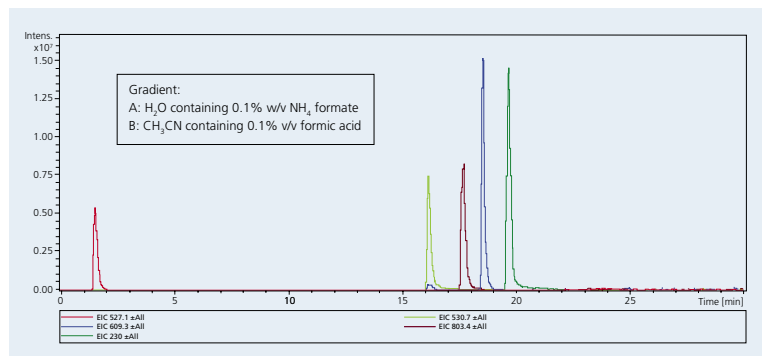
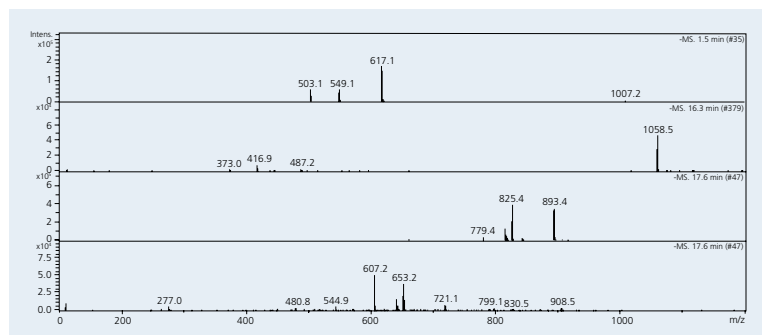


Figure 8 Mass spectra of test compounds in negative ion mode shown in Figure 6



ammonium formate in gradients with either pure acetonitrile or with acetonitrile spiked with 0.1% formic acid (FA). This latter combination functions as kind of “on the fly buffering,” which significantly affects the separation and ionization, although the apparent pH differences are not that dramatic.

Table 3 pH change during gradient of acetonitrile against ammonium formate (aqueous component: 0.1% w/v ammonium formate in water)

% aq.	% CH ₃ CN	pH CH ₃ CN	pH CH ₃ CN / 0.1% FA
100	0	6.3	6.3
50	50	6.9	6.7
10	90	7.5	7.1

Figure 5 shows the test mix separation using a gradient between 0.1% ammonium formate and pure acetonitrile. Under these conditions detection in negative ion mode is also possible, which often results in a more specific and less noisy signal (**Figure 6**). In **Figure 7** perfect resolution is achieved when using water with 0.1% w/v ammonium formate and acetonitrile with 0.1% v/v formic acid.

An interesting observation worthy of discussion are the mass spectra of the test components obtained in negative ion mode (**Figure 8**). The normal molecular ion is [M-H]⁻, 503.2 for raffinose, 779.4 for digoxin, 607.3 for reserpine and 1058.6 for bradykinin. In this case only the singly charged molecular ion is observed for the peptide bradykinin, contrary to positive ion mode, where the doubly charged ion is dominant. For the other test compounds addition of one formate anion, [M+45]⁻, is also observed.

In conclusion, the neutral volatile salts, ammonium acetate and ammonium formate, offer a much broader influence on analyte separation and ionization than do the acids. Their use, of course, is dictated by the particular LC-MS separation objectives or problems being addressed. Any limitations to their solubility may actually turn into the possibility of doing the separation or detection in a really unusual way.

References

- [1] “Mobile Phase Additives for LC-MS. Part 1: Acids – The Most Common Choice,” *Analytix* 2006/2, 8-9. (See also: “Mobile Phase Additives for LC-MS. Part 2: How to Overcome Suppression Effects of TFA,” *Analytix* 2006/3, 16-17. Both downloadable from: <http://www.sigma-aldrich.com/analytix>)
- [2] “Influence of solvent additive composition on chromatographic separation and sodium adduct formation of peptides in HPLC-ESI-MS”, Poster at HPLC 2006 San Francisco, June 2006; will appear in *J. Chromatogr. A*, symposium issue.

LPLC Resins and Media: A Comprehensive Collection Supported by Custom Solutions

..... Sigma-Aldrich offers a broad portfolio of adsorbent media and resins and customization for a wide variety of applications

Klaus Buckendahl, European Sales Development Manager ... KBuckend@europe.sial.com



Picture 1
Resins in different
package sizes

In the two most recent editions of the Analytix we presented innovative Lewatit® Mono Plus resins from LANXESS. Sigma-Aldrich also provides users with a comprehensive offering of other well-known resins and media for laboratory research and pilot-scale development. We offer a full line of off-the-shelf resins and media in manageable package sizes from leading manufacturers, including

- Dowex® Marathon®, Monosphere®, Optipore®, Retardion®, and other Dowex® resins (Dow Chemical)
- Amberlite®, Ambersep®, Amberlyst®, Amberjet®, Ambersorb®, Amberchrom®, Duolite® (Rohm and Haas)
- Diaion®, MCI-GEL®, Sepabeads® (Mitsubishi Chemical)
- Lewatit® (Lanxess)
- Sephadex®, (GE Healthcare)
- Supelpak™2 (purified Amberlite® XAD®-2), Supelite™ (Sigma-Aldrich)
- Toyopearl® (Tosoh Biosep)

These resins are available for all separation processes, including

- Anion & Cation Exchange
- Mixed Bed Ion Exchange
- Chelating Ion Exchange
- Nuclear Ion Exchange
- Adsorption
- Gel filtration



Picture 2 + 3
Rezoriant™ A161
cartridges (top) and
Porozorb™ cartridges
(bottom).

The resins portfolio is complemented by a broad range of inorganic adsorption and separation media for purification and preparative work. Some products within this line include

- Silica, unmodified, e.g. Davisil® (W.R. Grace) and E. Merck silicas, among others
- Silica, modified, e.g. C18, C8, aminopropyl, chloropropyl, etc.
- Florisil® magnesium silicate (U. S. Silica)
- Activated alumina
- Ambersorb® Carbons (Rohm and Haas)
- Celite® and CAFA II (Celite Analytical Filtration Aid)
- Carboxens carbon molecular sieves (Sigma-Aldrich)

Custom resin and media processing

In addition to the stock products listed above, Sigma-Aldrich, through its Supelco brand, offers custom-packaged quantities and processing to meet specific customer requirements. Following are just a few of the resin and media processing capabilities we offer.

Selection – Use our technical expertise to help you choose the right media and processing for your application.

Cleaning – Leachates from raw resins are a significant source of contamination. Our chemists can clean resins using a variety of solvent and solvent-free methods.

Pre-wetting – Dried media is often difficult to handle. We can pre-wet the media with solvents or water, making it compatible with your process and easier to handle with less of the media ending up on your laboratory bench and floor.

Blending – Some processes require mixed bed ion exchange resins. We can blend different resins, eliminating the need for you to do the mixing and keep two or more different resin stocks in your laboratory.

Drying – Residual moisture can interfere with certain analyses or reduce the resin's capacity or ability to disperse in organic solvents. We can dry nearly any resin to the moisture specifications your application requires.

Ionic form conversion – If an ion-exchange media is supplied by the manufacturer with a counterion that is not ideal for your application, we can convert it and supply the media in the correct ionic form.

Sanitizing and sterilizing – We can assure sterility according to USP methods and test for endotoxins. Many of our sterilization and sanitation processes are customer-defined.

QC testing, including C of A – Supelco is an ISO-9001:2000 registered vendor. As such, we take testing and certification very seriously. We test and certify the resins to meet your specific criteria, using a variety of analytical methodologies.

Custom tube-filling, packaging and package sizes – Bulk media or media that we custom process can be supplied to you in a nearly limitless format, from simple storage containers and in-line cartridges to process-specific devices. Porozorb™ (250, 1000 and 4000 mL) and Rezoriant™ A161 (1 and 5 mL) cartridges are examples of specially packaged LPLC media in column/cartridge format. If required, we have cleanroom facilities available for the packaging operation.

For more information on the media and resins with regards to selection, composition and application or to inquire about custom processing, please consult the Resins & Media section of the Supelco catalog, visit our web site www.sigma-aldrich.com or call the technical service of your local Sigma-Aldrich office.

VersaFlash™

High Throughput Flash Purification

It's in the particles!

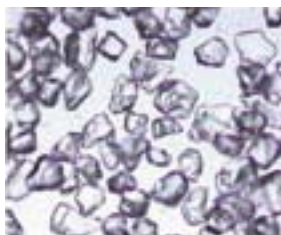
Irregular silica particles are most commonly used as packing materials for Flash Chromatography. However, compared to spherical particles, irregular shaped particles pack in a less uniform manner and give lower column efficiency. Irregular particles also generate fines that can cause changes in bed density, channeling and band broadening, in addition to possibly contaminating preps and fouling the system. Broad sample bands give rise to dilute sample fractions, which consume more solvent and take longer to rotovap.

VersaPak™ cartridges eliminate the problems associated with irregular particles. Packed with spherical particles, VersaPak™ cartridges have a denser, more evenly-packed bed. Fines are eliminated as a source of channeling, fouling and band-broadening. Sample components elute in sharper bands and more concentrated fractions reducing solvent consumption and evaporation time. The denser bed in VersaPak™ cartridges also gives higher sample capacity compared to the same volume packed with irregular particles.

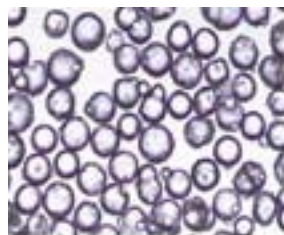
The savings are in the particles:

- Save Solvent – by more concentrated bands/fractions
- Save Time – by faster more efficient separations with less rotovap time
- Save Money – use smaller cartridge sizes and less solvent

Irregular Silica



Spherical Silica

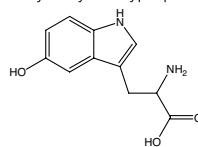


The VersaFlash™ High Throughput Flash Purification system is a versatile purification system that can cover a broad range of sample sizes. Cartridge sizes range from 11g (23mm ID) to 1900g (110mm ID) that can be used on the same system.

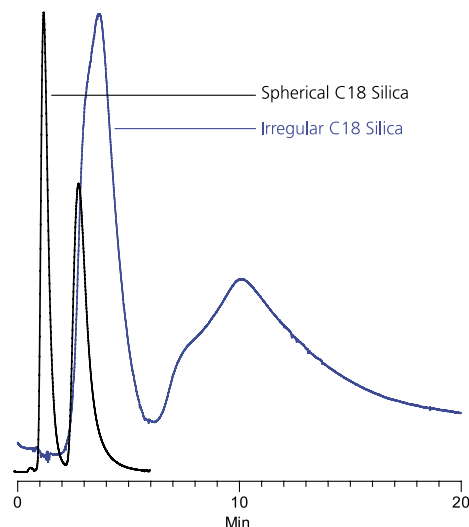
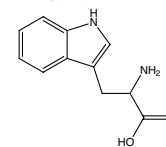


Improved efficiency on cartridges packed with spherical vs. irregular silica particles

5-hydroxy-DL-tryptophan



DL-tryptophan



For further information please request the VersaFlash™ Brochure (FWL) or visit our website: www.sigma-aldrich.com/versafash



NEW Adaptors allow you to use VersaPak™ Cartridges in Isco Flash Systems

SUPELCO

Analysis of Difficult Polar Compounds using Fluka Ion Pair Reagents and Supelco Ascentis HPLC Columns The proper selection and quality of reagent, its purity and high carbon load HPLC columns are critical to the success of HPLC separations employing IPC

Hansjörg Tinner, Scientist Quality Assurance ... htinner@europe.sial.com



Because reversed-phase HPLC is primarily dependent on hydrophobic interactions between the stationary phase and analyte, ion pairing is occasionally necessary to obtain sufficient retention of polar, ionizable compounds. Although there is debate over the precise mechanism, ion pair reagents ultimately enhance retention by increasing the amount of time the analyte spends in the stationary phase over the mobile phase. In addition to enhancing retention, ion pair reagents can also be used to change the selectivity, improve analyte solubility and suppress unwanted interactions, like adsorptive processes in silica-based particles.

The **choice** of ion pair reagent depends on the analyte, mobile phase conditions, detection method, and how much retention increase is desired. When using ion pairing, the mobile phase pH should allow at least partial ionization of the analyte molecules. Most conventional ion pair reagents are strong acids or bases, so pH does not influence their ionization state within the normal HPLC operating pH range. Counterions should be compatible with the mobile phase and analytes.

The **quality** of ion pairing reagents is also a very important consideration. Impure reagents can cause noisy or drifting baselines and irreproducibility. Insoluble matter can foul columns and sensitive instrument components. For many years, Fluka's ion pair reagents have represented the highest quality in a wide range of available chemistries (see **Table 1**).

Table 1 Product Listing

Fluka Ion Pair Reagents*

Cat. No.	Description	Package Size
74882	1-Octane sulfonic acid, sodium salt, monohydrate	2.5 g, 10 g, 50 g
86853	Tetrabutyl ammonium hydrogen sulfate	2.5 g, 10 g, 50 g

*IPC reagents used in this study. Many other Fluka brand ion pair reagents are available. Please see www.sigma-aldrich.com/ipc for the complete product listing.

Supelco Ascentis HPLC Columns*

Cat. No.	Description
581324-U	Ascentis C18 column, 15 cm x 4.6 mm, 5 µm
581373-U	Ascentis C18 Guard column kit, 2 cm x 4.0 mm, 5 µm (Holder and one replaceable cartridge)

*Ascentis columns used in this study. Other Ascentis phases and column dimensions are available. Please see www.sigma-aldrich.com/ascentis for the complete product listing.

They are subject to extensive application-specific QC testing, including:

- Filter test
- UV-absorption test
- Test for the absence of redox traces with a cyclic voltammogram
- HPLC gradient test

In this article, we will demonstrate the suitability of the Fluka ion pair reagents for RP-HPLC of three classes of polar, small molecule analytes: tetracyclines, vitamin B and phosphorylated compounds. Since the analytes were both polar and silanophilic, a Supelco Ascentis C18 HPLC column, which has a high carbon load of 25% for increased retention capability and an inert surface for symmetrical peaks, was chosen to complement the benefits of the Fluka-brand ion pair reagents.

Tetracyclines

Tetracyclines are broad-spectrum antibiotics prepared from the cultures of certain *Streptomyces* species. Their analysis by RP-HPLC is difficult because of the presence of polar hydroxyl, carbonyl, and amine groups (see **Figure 1**).

Without ion pair additives, retention and resolution is inadequate. However, by adding an anionic ion pair additive, in this case 1-octane sulfonic acid, retention is significantly increased and resolution is greatly improved (**Figure 2**). The pH of the mobile phase was important; above pH 5, chlortetracycline and doxycycline could not be separated and fronting was observed. Alkylsulfonate derivatives are in general applied under strong acidic conditions, which is in line with the obtained results. However, tetracyclines, particularly chlortetracycline, are not stable below pH 2 as they transform into dehydro derivatives. However, this degradation did not occur during the short duration of the HPLC analysis reported here.

Vitamin B compounds

These low molecular weight, polar N-containing compounds (**Figure 1**) are ionized below pH 7 with concomitant poor RP retention and peak shape. As with the tetracyclines, 1-octane sulfonic acid was added to the mobile phase to enhance retention (**Figure 3**). The pH of 2.1 ensured ionization of the amine group. Note the excellent peak shape provided by the Ascentis C18 column.

Figure 1
Representative
structures

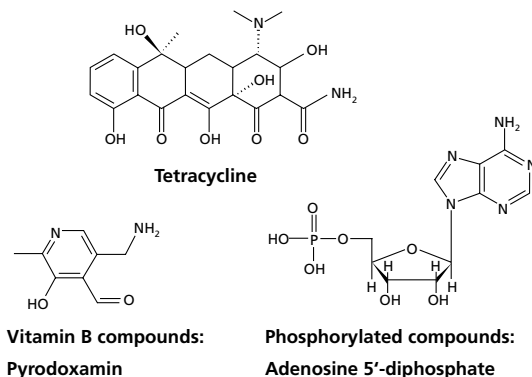
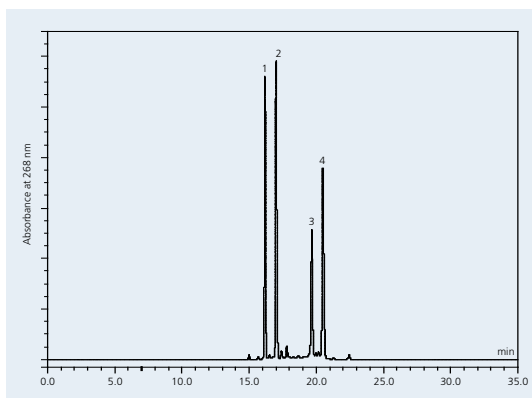


Figure 2
Separation of
tetracyclines on Supelco
Ascentis C18 by IPC
with Fluka 1-octane
sulfonic acid

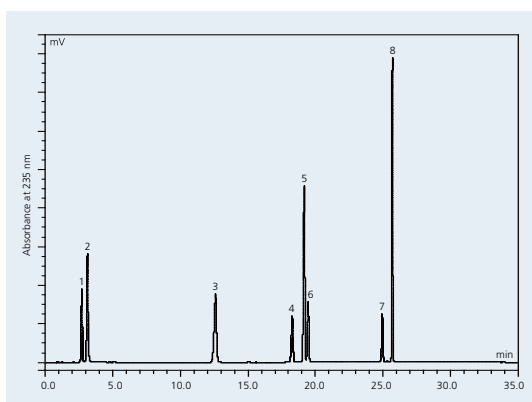


Conditions: column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 μ m particles (581324-U); mobile phase: (A) Acetonitrile; (B) 1.2 g/L 1-octanesulfonic acid in 10mM H_3PO_4 , pH 2.1; flow rate: 1.5 mL/min.; temp.: ambient (~25 °C)

Gradient: t=0 min, 5% A, 95% B; t=15 min, 15% A, 85% B; t=20 min, 35% A, 65% B.

Elution order: 1) Oxytetracycline; 2) Tetracycline; 3) Chlortetracycline; 4) Doxycycline

Figure 3
Separation of vitamin B
compounds on Supelco
Ascentis C18 by IPC
with Fluka 1-octane
sulfonic acid

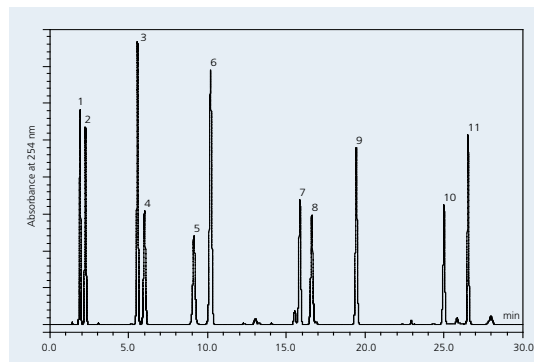


Conditions: column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 μ m particles (581324-U); mobile phase: (A) Acetonitrile; (B) 1.2 g/L 1-octanesulfonic acid in 10 mM H_3PO_4 , pH 2.1; flow rate: 1.5 mL/min.; temp.: ambient (~25 °C)

Gradient: t=0 min, 4% A, 96% B; t=10 min, 8% A, 92% B; t=20 min, 20% A, 80% B; t=25, 30% A, 70% B

Elution order: 1) Pyridoxal-5'-phosphate; 2) Cocarboxylase (thiamine diphosphate); 3) Nicotinamide; 4) Pyridoxal; 5) Thiamine monophosphate; 6) Pyridoxine; 7) Pyridoxamine; 8) Thiamine

Figure 4 Separation of phosphorylated compounds on Supelco Ascentis C18 by IPC with Fluka tetrabutylammonium sulfate



Conditions: column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 μ m particles (581324-U); mobile phase: (A) Acetonitrile; (B) 1.7 g/L tetrabutyl ammonium hydrogen sulfate in 10 mM Na_2HPO_4 , pH 7.0; flow rate: 1.5 mL/min.; temp.: ambient (~25 °C)

Gradient: t=0 min, 1% A, 99% B; t=11 min, 10% A, 90% B; t=16 min, 20% A, 80% B; t=25, 40% A, 60% B

Elution order: 1) Cytidine-5'-diphosphocholine; 2) Thiamine monophosphate; 3) beta-Nicotinamide adenine dinucleotide (NAD); 4) Cocarboxylase (thiamine diphosphate); 5) Uridine-5'-diphosphoglucose, 6) Adenosine-5'-monophosphate; 7) beta-Nicotinamide adenine dinucleotide reduced (NADH); 8) Pyridoxal-5'-phosphate; 9) Adenosine-5'-diphosphate; 10) Coenzyme A; 11) Crotonoyl-coenzyme A

Phosphorylated compounds

Phosphorylated compounds (**Figure 1**) are prevalent in biochemical analyses. To achieve RP retention and separation, tetrabutyl ammonium bisulfate at pH 7.0, which interacts with the phosphate group, was added to the mobile phase (**Figure 4**). Because pyridoxal-5'-phosphate, thiamine monophosphate and cocarboxylase contain both amine and phosphate groups, they can accommodate both anionic and cationic ion pair reagents. Note that **Figures 3 and 4**, both of which contain these compounds, show a peak order reversal with the two different reagents. This indicates that the retention mechanism is influenced by more than just compound polarity. It also reinforces the fact that the choice in IPC reagent can be leveraged in order to optimize the separation.

Conclusion

The combination of Fluka ion pair reagents and Supelco Ascentis columns is an excellent solution for the analysis of highly polar, silanophilic compounds by RP-HPLC. The combination leverages the power of RP-HPLC to differentiate between closely-related molecules and the power of ion pairing to enhance retention and selectivity. Using the ion pairing method described here, difficult to analyze polar compounds were successfully separated with adequate retention and resolution, but without tailing, fronting or co-elution.

Well-Defined pI Markers and Convenient Buffers for Gel and Capillary IEF Improve the accuracy and sensitivity of IEF and CIEF with both UV and fluorescence detection using synthetic pI markers from Sigma-Aldrich

By Pierre Nording, Product Manager Biochemistry ... pnording@europe.sial.com

Isoelectric focusing (IEF), the separation of amphiphilic analytes, especially proteins, according to their isoelectric point, is an important electrophoretic technique. It complements other separation methods based on molecular size or hydrophobicity. Gel electrophoresis, the common technology for IEF, minimizes convection and introduces an additional gel-sieving effect to separate proteins by size. However, slab gel-based IEF

has disadvantages, including long analysis times, limited resolution, difficult detection and poor adaptability to automation.

In contrast, performing IEF using electrophoresis in capillaries (CIEF) can overcome these drawbacks, permitting more rapid analyses, greater resolution and the ability to automate. CIEF separations are carried out in fused-silica capillaries of just 25 – 75 µm internal diameters. The electrophoresis takes place in free solution and convection currents are controlled by thermostating. After focusing is complete the solutes are pumped out of the capillary and detected. UV detection is the most popular method, but UV-induced fluorescence emission is also used to increase sensitivity of dansylated or fluorescamine, o-phthalaldehyde or coumarin-derivatized proteins.

Table 1 Fluorescent IEF-Markers and protein markers

Cat. No.	Description	pI	Fluorescence	
			Em _{max} [nm]	Exc. [nm]
35096 74169	solid stock solution	2.1	430	340
17952 72172	solid stock solution	3.0	440	360
17953 40677	solid stock solution	3.5	415	318
17954 89827	solid stock solution	4.0	415	310
17955 89149	solid stock solution	4.5	424	336
17956 89478	solid stock solution	5.1	415	330
17957 77866	solid stock solution	5.5	412	325
17958 73938	solid stock solution	6.2	500	394
17959 73376	solid stock solution	6.6	500	396
17961 89508	solid stock solution	6.8	418	338
17962 89951	solid stock solution	7.2	500	387
17963 89952	solid stock solution	7.6	495	385
17964 75734	solid stock solution	8.1	420	340
17966 89357	solid stock solution	8.7	500	390
17967 90699	solid stock solution	9.0	495	385
46276 89268	solid stock solution	9.5	415	325
17968 77672	solid stock solution	10.3	495	388
17951	stock solution of Marker-Mix	-	-	-
56730	Mixture of 7 proteins, lyophilised	3.6-6.6		
56733	Mixture of 8 proteins, lyophilised	3.6-9.3		

Typical Analysis Conditions for CIEF Using Pressure Mobilization

Capillary: Neutral capillary
 Anolyte: 91 mM phosphoric acid in gel buffer
 Catholyte: 20 mM sodium hydroxide in water
 Detection: UV at 280 nm
 Temperature: 20 °C
 Injection: 20 psi for 1 min.
 Polarity: Inlet anode, outlet cathode
 Focusing voltage: 500 V/cm
 Focusing time: 2 min.
 Mobilization: 0.5 psi, 500 V/cm anolyte to catholyte (mobilization should be stopped after the last marker is eluted to avoid the filling of the capillary with anolyte)

Benefits of Synthetic pI Markers

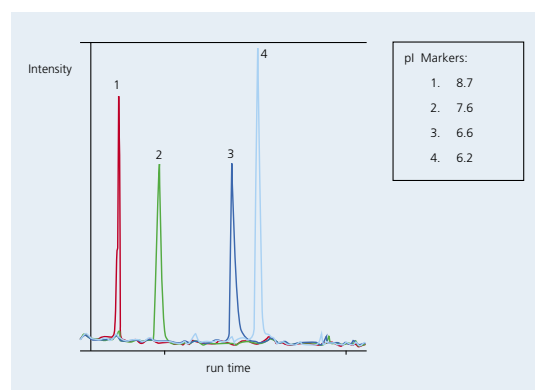
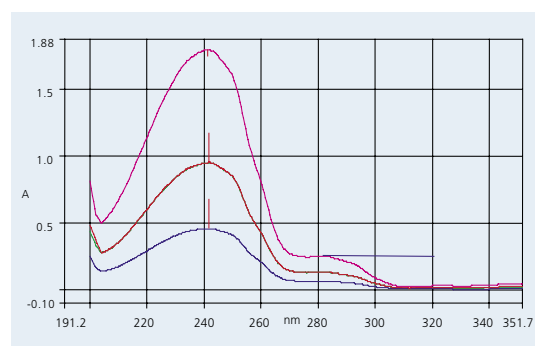
Whether the IEF occurs in slab gels or capillaries, standardization of results is important for reliable protein identification. Typically, protein standards are used, but they have inherent disadvantages including limited stability, varying levels of impurities and lot-to-lot inconsistency.

To overcome problems associated with protein standards, Sigma-Aldrich has developed and introduced **synthetic pI markers (Table 1)** which can be used for both UV absorption and fluorescence detection. The maximum absorbances of the individual markers are between 308 and 350 nm. For fluorescence detection, an excitation wavelength of 310 nm (individual excitation maximum:

Table 2 Ready-to-use buffers for HPCE

Cat.No.	Product Name
82609	Buffer Solution pH 2.5 for HPCE [20 mM citric acid-NaOH]
82581	Buffer Solution pH 2.5 for HPCE [20 mM sodium citrate]
82582	Buffer Solution pH 3.0 for HPCE [20 mM sodium citrate]
82621	Buffer Solution pH 3.0 for HPCE [Cupric electrolyte buffer pH 3.0]; special quality for the detection of cations (alkali and alkaline earth metals and amines)
82622	Buffer Solution pH 3.0 for HPCE [150 mM potassium phosphate]
82582	Buffer Solution pH 3.0 for HPCE [20 mM sodium citrate]
82583	Buffer Solution pH 3.5 for HPCE [20 mM sodium citrate]
82584	Buffer Solution pH 4.0 for HPCE [20 mM sodium citrate]
82585	Buffer Solution pH 4.5 for HPCE [20 mM sodium citrate]
82586	Buffer Solution pH 5.0 for HPCE [20 mM sodium citrate]
82587	Buffer Solution pH 5.5 for HPCE [20 mM sodium citrate]
82588	Buffer Solution pH 6.0 for HPCE [20 mM sodium citrate]
82589	Buffer Solution pH 6.5 for HPCE [20 mM sodium phosphate]
82614	Buffer Solution pH 7.0 with SDS f.HPCE [100 mM boric acid/50 mM sodium phosphate, with 50 mM SDS]; special quality for Micellar Electrokinetic Capillary Chromatography (MECC)
82591	Buffer Solution pH 7.0 for HPCE [20 mM sodium citrate]
82636	Buffer Solution pH 7.0 for HPCE [50 mM sodium citrate]
82637	Buffer Solution pH 7.0 for HPCE [100 mM sodium citrate]
82592	Buffer Solution pH 7.5 for HPCE [20 mM sodium phosphate]
82619	Buffer Solution pH 7.7 for HPCE [Pyromellitic acid electrolyte buffer pH 7.7]; special quality for the detection of inorganic and low molecular weight organic acid anions
82593	Buffer Solution pH 8.0 for HPCE [20 mM sodium phosphate]
82594	Buffer Solution pH 8.0 for HPCE [20 mM sodium tetraborate]
82615	Buffer Solution pH 8.0 with methylcellulose for HPCE [50 mM TRIS-borate/ 2.5 mM EDTA, 0.5% methylcellulose]; special quality for separation of DNA restrictions fragments
82633	Buffer Solution pH 8.0 for HPCE [50 mM sodium borate]
82634	Buffer Solution pH 8.0 for HPCE [100 mM sodium borate]
82601	Buffer Solution pH 8.5 for HPCE [20 mM sodium phosphate]
82602	Buffer Solution pH 8.5 for HPCE [20 mM sodium tetraborate]
82616	Buffer Solution pH 8.6 with urea for HPCE [100 mM TRIS/100 mM boric acid/2 mM EDTA/7 M urea]; special quality for separation of nucleic acids
82603	Buffer Solution pH 9.0 for HPCE [20 mM sodium phosphate]
82604	Buffer Solution pH 9.0 for HPCE [20 mM sodium tetraborate]
82605	Buffer Solution pH 9.5 for HPCE [20 mM sodium phosphate]
82606	Buffer Solution pH 10.0 for HPCE [20 mM CAPS]
82607	Buffer Solution pH 10.5 for HPCE [20 mM CAPS]
82608	Buffer Solution pH 11.0 for HPCE [20 mM CAPS]
82617	Buffer Solution pH 11.0 for HPCE [20 mM glycine-NaOH]
84428	Hydrochloric acid Solution for HPCE
72079	Sodium hydroxide Solution for HPCE
95283	Water for HPCE

310 to 400 nm) is suggested; the emission maximum of the individual markers lies between 410 and 500 nm. An example of the CIEF of four synthetic pI markers is shown in **Figure 1**. The UV absorbance spectra of three concentrations of pI 7.6 marker can be found in **Figure 2**.

Figure 1 Example of CIEF with Sigma-Aldrich fluorescent pI Markers**Figure 2** UV spectrum of Sigma-Aldrich fluorescent IEF pI marker 7.6 at three different concentrations (UVmax and absorbance at UVmax shown)

For Your Convenience: Buffers for HPCE

We also offer a complete range of ready-to-use buffers for high performance capillary electrophoresis, including CIEF (**Table 2**). These buffers cover the pH range from 2.5 to 11 and meet the quality requirements of HPCE.

The guaranteed characteristics for each reagent include:

- No insoluble impurities. The HPCE buffers are filtered through a 0.2 µm filter membrane after production.
- Minimal absorption over a wide wave length range
- Virtually no fluorescent impurities
- Application-tested

Reference

Horka, M.; Willmann, Th.; Blum, M.; Nording, P.; Friedl, Z.; Slais, K. Capillary isoelectric focusing with UV-induced fluorescence detection, *J. of Chromatography A* **2001**, 916, 65 - 71.

Selectophore® Ionophores Highly pure and stable compounds for the preparation of ion selective electrodes and optical sensors

By Michael Jeitziner, Market Segment Manager Analytical Reagents ... mjeitziner@sial.com

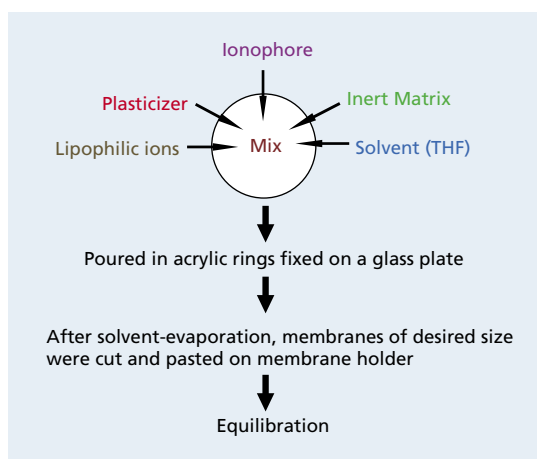
Introduction

Chemical sensors are devices that can quantitatively and reversibly measure a particular analyte. Due to their specificity and sensitivity, they are becoming more and more prevalent in chemical analysis, environmental monitoring, medicine and food analysis, among other areas. Many chemical sensors have been developed and commercialized in the past few decades, with more under development to keep pace with new analytical challenges.

Chemical sensors are often used in an electrode format which is then immersed in the sample to be analyzed. Presence of the target analyte is evidenced by a change in the electrode potential. The principle of the membrane preparation for ion-selective electrodes (ISE) is to incorporate an organic compound as the ionophore into a polymer (e.g. polyvinyl chloride) membrane together with an appropriate plasticizer and additive which provide the membrane with the properties of a liquid phase (see **Figure 1**). This approach can also be used to prepare optical sensor membranes.

Figure 1

Preparation of an ion-selective membrane for potentiometric sensors



Selectophore® Grade Ionophores

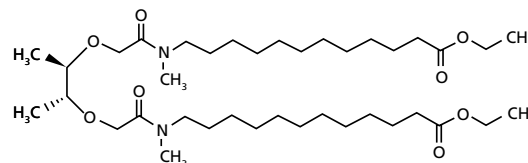
Selectophore® from Sigma-Aldrich is the most comprehensive product line available to fulfill requirements for the preparation of sensor membranes for ion-selective potentiometric and optical devices. Ionophores and auxiliary components (polymers, plasticizers, additives) used in potentiometric or optical sensors have different specifications than materials used for other applications. It is essential that the product is free of disrupting impurities, such as metal ions or surfactants. However, the specific purity requirements

are a function of the analyte, with some analyte-ionophore combinations more sensitive to impurities than others. For this reason, all Selectophore® ionophores are application tested.

For complete list of our Selectophore® product range, please visit the web site: www.sigma-aldrich.com/sensoric.

Note: Patents for synthesis or applications may apply.

Selectophore, Calcium Ionophore I (ETH 1001)



ETH 1001, CAS# 58801-34-6

Function-tested, $\geq 99.0\%$ (HPLC)

Cat. No. 21192 (Fluka)

Calcium Ionophore I, also known as ETH 1001, is a neutral ionophore with extremely high selectivity for Ca^{2+} ions. The synthesis and purification of Calcium Ionophore I requires a great deal of expertise. Over the last two decades, Sigma-Aldrich researchers have improved the synthesis and purification of this compound and can now offer Calcium Ionophore I with purity and performance unmatched in the market. The recent synthesis optimization resulted in a highly stable product with shelf life exceeding four months.

Characteristics:

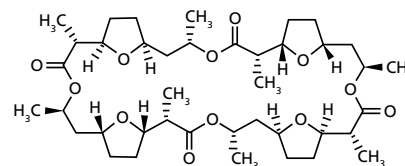
Linear range: 2×10^{-7} to 1×10^{-1} mol/L (CaCl_2)

Slope (sensitivity): 28 mV/dec

Detection limit: 1×10^{-7} mol/L

Shelf life: >4 months

Selectophore, Ammonium Ionophore I (nonactin)

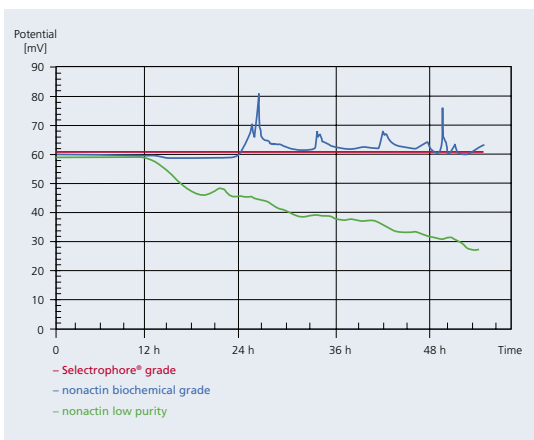


Nonactin, CAS# 6833-84-7

Function-tested

Cat. No. 09877 (Fluka)

Figure 2
Comparison of different quality grades of nonactin.
Source: Nadler Technologies, Switzerland (www.nadler.ch)



Ammonium Ionophore I is an antibiotic isolated from fermentation. Although this ionophore is very selective for NH_4^+ ions, it can also be used for the determination of urea after enzymatic decomposition.

Characteristics:

- Linear range: 1×10^{-6} to 1×10^{-1} mol/L (NH_4Cl)
- Slope (sensitivity): 60.8 mV/dec
- Detection limit: 6×10^{-7} mol/L
- Shelf life: >4 months

The example in **Figure 2** illustrates the advantage of the Selectophore, brand over other quality grades. Although Ammonium Ionophore I is simply the compound nonactin, there are enormous differences in the quality derived from various sources. Competitive products often contain ionic impurities, which leach out over a period of time causing anomalous baselines.

Product List Selected Ionophores from Sigma-Aldrich*

Cat. No.	Brand	Description	Package Size
21192	Fluka	Calcium Ionophore I (ETH 1001), Selectophore®	50 mg, 250 mg
09877	Fluka	Ammonium Ionophore I (nonactin), Selectophore®	50 mg, 250 mg

*For the complete list of our Selectophore® products, please visit www.sigma-aldrich.com/sensoric.

NEW Ionophore Web page



Use our interactive sensorics Web page to find the right Selectophore® product for your particular application.

Move the pointer over the Periodic Table to see the data sheets for the ionophores we offer for each element. You can also follow the links below the Periodic Table to view our complete list of Selectophore® products and information about our equipment for pH and ion-selective electrodes and the preparation of optode membranes. The Web page is frequently updated with the newest ionophores from Sigma-Aldrich.

www.sigma-aldrich.com/sensoric

Diagnostic Media and Supplements for *Listeria monocytogenes* Chromogenic media and other products for the selective growth and identification of this important food-borne pathogen

By Jvo Siegrist, Product Manager Microbiology ... isiegris@sial.com

In 2005, *Listeria* contamination prompted two prominent food product recalls in Europe and the US. In June, a *Listeria* outbreak in Switzerland resulted in two deaths, two miscarriages and sickened a number of people who consumed Tomme cheese, a soft cheese made from raw milk in which *Listeria* can flourish [1]. In August, ice cream from a US producer was recalled when *Listeria* was detected by routine government health inspection tests [2]. Fortunately, no illnesses or deaths were linked to that contamination. These events were not isolated; in 1987 an outbreak of *Listeria* from contamination of another Swiss soft cheese caused 30 deaths and multiple miscarriages [1]. Obviously, control of this pathogen at the source by food producers and its reliable detection in finished products are of utmost public safety concern [3].

The pathogen behind these outbreaks is the bacillus *Listeria monocytogenes*, a Gram-positive, non spore forming, rod-shaped flagellate. It is ubiquitous: it exists in plants and soil as well as in the guts of birds, fish,

shellfish and some mammals, including humans. Some studies suggest that 1-10 % of humans may be intestinal carriers of *L. monocytogenes*.

Consuming contaminated foods or handling infected farm animals can cause the disease listeriosis [4], which is characterized by mild, flu-like symptoms. Infection can be serious when it occurs in the very young or very old, those with suppressed immune systems and pregnant women. Foods associated with the spread of *Listeria* include:

- unpasteurized (raw) dairy products
- soft cheeses
- raw vegetables
- raw and smoked fish
- processed cold meats such as pate

Its resilience is at the heart of *Listeria's* problems from a food safety standpoint. While the common belief is that

Figure 1

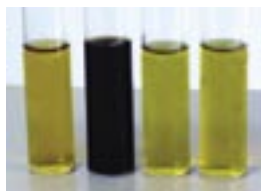
ISO Protocol
(EN-ISO 11290-1:1996)
for detection and
enumeration of *Listeria
monocytogenes*



Figure 2

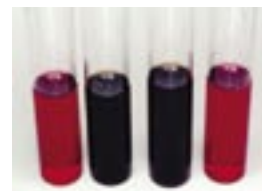
Fraser Broth Fluka 69198

1. Control,
2. *Listeria monocytogenes* (ATCC 29112),
3. *E. coli* (ATCC 25922),
4. *E. faecalis* (ATCC 29262)

**Figure 3**

PALCAM Listeria Selective

- Enrichment Broth, Vegitone Fluka 59859; 1. Control, 2. *Listeria monocytogenes* (ATCC 19112), 3. *Listeria ivanovii* (ATCC 19119), 4. *Staphylococcus aureus* (ATCC 29262)

**Figure 4***Listeria mono*Differential Agar
Fluka 77408*Listeria innocua*

(ATCC 33090) (left)

Listeria monocytogenes

(ATCC 19112) (right)

**Figure 5***Listeria mono* Confirmatory

Agar Fluka 92302

In front *Listeria monocytogenes*

refrigeration prevents growth of food-borne pathogens, *Listeria* flourishes at refrigerator temperatures. It is also resistant to acidic and basic conditions. Although its optimum pH range is 5 to 9.6, it survives outside this range as well.

The reliable verification of the presence or absence of *Listeria* is an important food safety challenge. Analytical microbiology leverages a microbe's specific biochemical or physical traits to accomplish its selective growth and confirm its presence or absence in the suspect food. Often it is necessary to perform multiple tests for confident determinations.

The biochemical profile of *Listeria* includes: catalase positive, oxidase negative, fermentation of carbohydrates to acid but not to gas, hydrolysis of esculin and sodium hippurate, methyl red positive, ammonia production from arginine, negative reaction for hydrogen sulfide production, indole negative, nitrate reductase negative, no gelatin liquefaction, no hydrolysis of starch and no

urea hydrolysis. Sigma-Aldrich biochemists continuously develop innovative products and commercialize them through the Fluka Brand. A list of reliable diagnostic tests for many pathogens, including *Listeria*, is displayed in **Table 1**.

EN ISO 11290-1:1996/A1:2004 and EN ISO 11290-2:1998/A1:2004 (Microbiology of Food and Animal Feeding Stuffs) describe a horizontal method for the detection and enumeration of *Listeria monocytogenes*. A flow chart of the process appears in **Figure 1**. The method involves a general four-step process: enrichment, identification, isolation and confirmation. The *Listeria* Diagnostic tests from Sigma-Aldrich contain the elements necessary to selectively grow and identify *Listeria* in food substances according to this methodology. These media, tests and related products are described in **Table 1**.

These and other products for microbiology can be found at our web site: www.sigma-aldrich.com/microbiology

Table 1 Sigma-Aldrich diagnostic products for *Listeria***Selective Enrichment Media**

Cat. No.	Brand	Description	Package Size
69198	Fluka	Fraser Broth, Base (see Figure 2)	500 g
18038	Fluka	Fraser Selective Supplement (use with 69198)	5 vials
90836	Fluka	Fraser Supplement (use with 69198)	10 vials
F6672	Sigma	Fraser secondary enrichment broth base	500 g
F2674	Sigma	Fraser enrichment supplement (use with F6672)	1 vial
L9410	Sigma	Fraser Listeria Supplement (use with F6672)	1 vial
62353	Fluka	Listeria Enrichment Broth according to FDA/IDF-FIL	100 g, 500 g
62351	Fluka	Listeria Selective Supplements according to IDF-FIL (use with 62353)	16 vials
62348	Fluka	Listeria Selective Supplement according to FDA (use with 62353)	16 vials
59859	Fluka	PALCAM Listeria Selective Enrichment Broth, Vegitone (see Figure 3)	500 g
03396	Fluka	PALCAM Listeria Selective Supplement according to Van Netten et al. (use with 59859)	16 vials
94485	Fluka	UVM Listeria Selective Enrichment Broth, modified	100 g, 500 g

Identification Media

Cat. No.	Brand	Description	Package Size
62355	Fluka	Listeria Selective Agar	100 g, 500 g
62653	Fluka	LPM Agar	100 g, 500 g
43963	Fluka	Moxalactam Supplement (use with 62653)	5 vials
63595	Fluka	McBride Agar	100 g, 500 g
77850	Fluka	(+/-)-1-Phenylethanol (use with 63595)	250 mL, 1 L
01810	Fluka	Cycloheximide (Actidione) (use with 63595)	1 g, 5 g
75805	Fluka	Oxford Agar	100 g, 500 g
75806	Fluka	Oxford-Listeria Selective Supplement (use with 75805)	13 vials
75977	Fluka	PALCAM Listeria Selective Agar	100 g, 500 g
15776	Fluka	PALCAM Listeria Selective Agar, Vegitone	500 g
03396	Fluka	PALCAM Listeria Selective Supplement according to Van Netten et al. (use with 75977 or 15776)	16 vials
93395	Fluka	Tryptone Soya Yeast Extract Agar	500 g

Confirmation Media

Cat. No.	Brand	Description	Testing features	Package Size
B1676	Sigma	Blood Agar Base No. 2	Lysis test	100 g, 500 g
07410	Fluka	Carbohydrate Consumption Broth	Fermentation ability	500 g
53707	Fluka	HiCrome™ Listeria Agar Base, modified	β-glucosidase activity, rhamnose fermentation	50 g, 250 g
59688	Fluka	HiCrome™ Listeria Selective Supplement	(use with 53707)	5 vials
92302	Fluka	Listeria mono Confirmatory Agar (Base) (see Figure 5)	Presence of phosphatidylinositol specific phospholipase C of Listeria monocytogenes and fermentation of α-methyl D-mannoside	38.5 g, 500 g
15895	Fluka	Listeria mono Enrichment Supplement II	(use with 92302)	5 vials
92301	Fluka	Listeria mono Selective Supplement I	(use with 92302)	5 vials
91603	Fluka	Listeria mono Selective Supplement II	(use with 92302)	5 vials
77408	Fluka	Listeria mono Differential Agar (Base) (see Figure 4)	Presence of phosphatidylinositol specific phospholipase C of Listeria monocytogenes	36 g, 500 g
03708	Fluka	Listeria mono Enrichment Supplement I	(use with 77408)	5 vials
92301	Fluka	Listeria mono Selective Supplement I	(use with 77408)	5 vials
91603	Fluka	Listeria mono Selective Supplement II	(use with 77408)	5 vials
55265	Fluka	Listeria Motility Medium	Motility test	500 g

Confirmation Tests

Cat. No.	Brand	Description	Testing features	Package Size
88597	Fluka	Catalase Test (H ₂ O ₂ , 3% solution)	Presence of catalase	100 mL
77730	Fluka	Gram Staining Kit	Cell wall properties	1 Kit
40405	Fluka	Hippurate Disks	Hydrolysis of hippuric acid	25 Disks
92472	Fluka	Hippurate Strips	Hydrolysis of hippuric acid	50 Strips
7345	Fluka	Oxidase Reagent acc. Gaby-Hadley A	Presence of oxidase	100 mL
7817	Fluka	Oxidase Reagent acc. Gaby-Hadley B	Presence of oxidase	100 mL
18502	Fluka	Oxidase Reagent acc. Gordon-McLeod	Presence of oxidase	100 mL
40560	Fluka	Oxidase Strips	Presence of oxidase	100 Strips
70439	Fluka	Oxidase Test	Presence of oxidase	50 Disks

References

- [1] *Listeria* outbreak under control. Neue Zürcher Zeitung AG, June 8, 2005.
- [2] U.S. Food and Drug Administration. http://www.fda.gov/oc/po/firmrecalls/lappert08_05.html (accessed May 2006).
- [3] Food-Borne Pathogenic Microorganisms and Natural Toxins Handbook: The "Bad Bug Book" U.S. FDA/CFSAN. 2003. Center for Food Safety and Applied Nutrition, Food and Drug Administration, College park, MD. <http://vm.cfsan.fda.gov/~mow/intro.html>
- [4] Cossart, P.; Bierne, H.; The use of host cell machinery in the pathogenesis of *Listeria monocytogenes*. Curr. Opin. Immunol. (England) 2001, 13(1), 96-103.

KOH and HCl Isopropanol Solutions for Determination of the Total Acid Number of Mineral Oils

By Michael Jeitziner, Market Segment Manager Analytical Reagents ... mjeitziner@sial.com



Oxidative aging of oils and lubricants produces caustic compounds that can damage leaded bearings and coatings that they contact. Acidic compounds in new and used oils include organic and inorganic acids, esters, phenols, lactones and resins. Basic compounds are also of concern, and include organic and inorganic bases, amines, salts of weak acids (like soaps) and salts of polyacidic bases. Salts of heavy metals and additives such as inhibitors and detergents can be either acidic or basic.

The acid content of the sample, which is an indicator of its age and quality of the sample, can be determined by

titration with KOH, giving the total acid number (TAN). Metrological agencies worldwide, including DIN, ANSI and ASTM, have established methodologies for the determination of TAN.

Certain oils, such as many cutting oils, rust proofing oils and other compounded oils, or excessively dark-colored oils that cannot be analyzed for acid number by this test method due to obscurity of the color-indicator end point, can be analyzed by ASTM D664. The determination of the TAN of trichlorotrifluoroethane and other halocarbons is measured according to ASTM D3444.

Sigma-Aldrich offers all reagents used for TAN determination. We recently introduced 0.1 M KOH and HCl solutions in isopropanol according to DIN 51558-1, ANSI/ASTM D974 and IP 139/65.

Cat. No.	Brand	Description	Package Size
35008	Riedel-de Haën	Potassium hydroxide, 0.1 M in isopropanol	1 L
35009	Riedel-de Haën	Hydrochloric acid, 0.1 M in isopropanol	1 L
33464	Riedel-de Haën	α -Naphtholbenzein, puriss p.a., Reag. Ph.Eur.	5 g
34576	Riedel-de Haën	Methyl Orange solution 0.1%, Reag. Ph.Eur.	500 mL, 1 L

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Sensitive trace analysis applications require extremely pure sample preparation reagents. Metal ions and other impurities must be absent or at vanishingly low levels.

To meet these stringent requirements, Sigma-Aldrich offers Fluka brand **TraceSELECT™** and **TraceSELECT™-Ultra** high purity acids, bases, water and salts designed specifically for trace analysis. All **TraceSELECT™** products are guaranteed 99.99% to 99.9999% pure.

TraceSELECT™Ultra

Designed for the ultra trace analysis level down to ppb and ppt. Typical metal trace impurities are below 0.1 $\mu\text{g}/\text{kg}$ (<0.1 ppb) and in some cases down to the ppq level. To maintain purity, most of **TraceSELECT™Ultra** products are delivered in PFA bottles.

TraceSELECT™

Designed for sample preparation and analysis in the ppm and ppb level. The blank values for metal traces are typically below 0.01 mg/kg (<0.01 ppm). For maximum reliability and confidence in your trace analysis determinations, rely on the high purity Fluka brand **TraceSELECT™** and **TraceSELECT™Ultra** reagents from Sigma-Aldrich.

For further information, please visit our web site:
www.sigma-aldrich.com/trace_analysis

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