

the Reporter

EUROPE

Volume 14 November 2004 International issue

SUPELCO

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HPLC/LC

Separations of Tetracycline Antibiotics by Reversed Phase HPLC

Sample Preparation

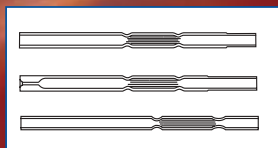
Determination of α - β Thujone and Related Terpenes in Absinthe using Solid Phase Extraction and Gas Chromatography

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SIGMA-ALDRICH

EDITORIAL

Dear Scientist,

Earlier this year we communicated our plans to improve our website. In May, significantly improved content for Supelco products became available. We hope the greater product detail has proved valuable to you and enhanced your overall experience visiting our website.

Many other improvements have occurred since May, specifically:

- Web Access to Support Documentation such as C of A's and MSDS's

In July, we deployed Certificates of Analysis for Supelco products. We have over five years of C of A data available through the website. Enter product and lot number, and you can access our C of A's.

We also have loaded MSDS information for over 80% of Supelco products. This information is available directly through the site, and we are working hard to load the MSDS information for the rest of our products.

- Improved Registration Process and Easier Access to Price and Availability

In August of this year, we deployed a new customer registration process for our website. It is easier to register, simpler to recover lost passwords or IDs, and allows you to directly manage your registration profile.

The new registration process also permits easier access to price and availability information. The shopping cart is available anywhere in the site. It therefore is faster, easier, and more convenient to create an order.

- More Change is on the Way

Among the changes you will see soon are increased use of images and interactive tools to enhance the product selection process, improved navigation for more direct access to products and technical information, and better use of profiles to make your experience within our site tailored to your needs.

We plan to continue to improve. Your recommendations and input about our site are invaluable in our improvement effort. Please, tell us about the improvements you would like to see. Stay with us, and keep checking back to www.sigma-aldrich.com for updates.

Sincerely,



David Henderson & Jo Ann Williams
Supelco Website Improvement Team



HPLC ARTICLE

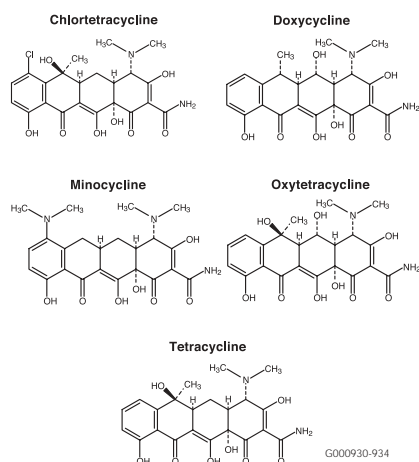
Separations of Tetracycline Antibiotics by Reversed Phase HPLC, Using Discovery Columns

There are more than 100 antibiotics on the market today, and many more are in development. Resistance to antibiotics is a significant problem (1,2): an antibiotic that takes a decade to bring to market can induce resistance within months of its introduction into clinical practice (3). The frequency of resistance in bacteria and the numbers of drugs to which they are resistant are increasing. Therefore, it is critical to monitor the level of antibiotics given to humans and animals.

HPLC is a powerful tool for isolation and quantification of antibiotics. In this application, five tetracycline antibiotics (Figure A) were analyzed by HPLC, using Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. Tetracycline antibiotics have a broad spectrum of activity, are relatively safe, and are effective against many infections caused by Gram-negative and Gram-positive bacteria (4,5).

Chromatographic separations were performed on a Waters Alliance HPLC system. All injections were made through an autosampler. A Waters 2487 dual wavelength UV detector was used to monitor the UV absorbance of samples at 260nm. The 15cm x 4.6mm ID Discovery C18, Discovery C8, and Discovery RP-AmideC16 reversed phase HPLC columns were used without guard columns or filters. The packing particles in all columns were 5µm in diameter.

Figure A. Structures of Tetracycline Antibiotics



Doxycycline, minocycline, tetracycline, chlortetracycline, and oxytetracycline were obtained from Sigma Chemical Co. All antibiotics were dissolved in 25mM KH₂PO₄ buffer, pH 3.

The five tetracycline antibiotics were separated by gradient elution. Column temperature was controlled at 35°C. Column pressure was below 1050psi in all cases. Detailed conditions for each analysis are presented with the corresponding chromatogram.

Figure B. Tetracycline Antibiotics on a Discovery C18 HPLC Column

Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 504955
 Mobile Phase: (A) 25mM KH₂PO₄, pH 3 (B) acetonitrile
 10% B to 50% B over 15 min
 Flow Rate: 1mL/min
 Pressure: <900psi
 Temperature: 35°C
 Detection: UV, 260nm
 Injection: 15µL 25mM KH₂PO₄, pH 3 containing 20µg/mL each analyte

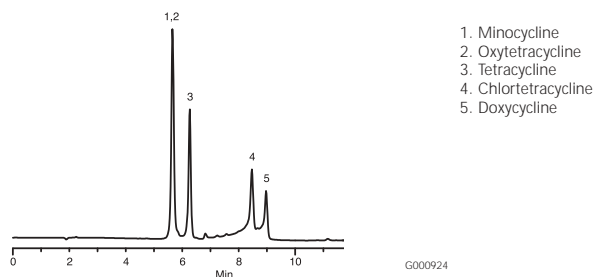


Figure C. Tetracycline Antibiotics on a Discovery RP-AmideC16 HPLC Column

Column: Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 505013
 Mobile Phase: (A) 25mM KH₂PO₄, pH 3 (B) acetonitrile
 10% B to 40% B over 15 min
 Flow Rate: 1mL/min
 Pressure: <950psi
 Temperature: 35°C
 Detection: UV, 260nm
 Injection: 20µL 25mM KH₂PO₄, pH 3 containing 20µg/mL each analyte

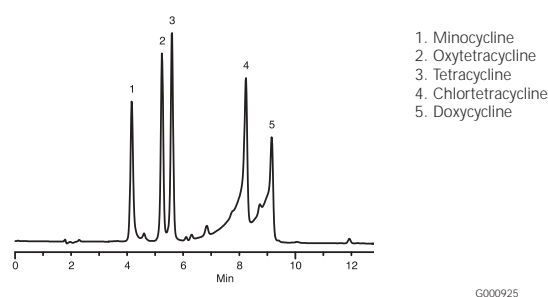
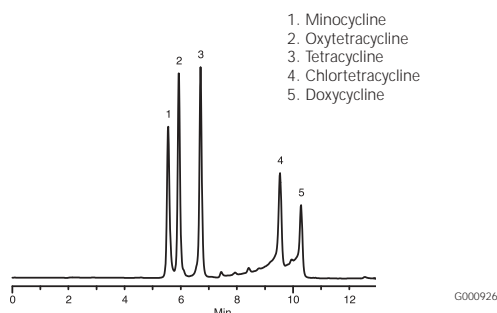


Figure D. Tetracycline Antibiotics on a Discovery C8 HPLC Column

Column: Discovery C8, 15cm x 4.6mm ID, 5µm particles
 Cat. No.: 59353-U
 Mobile Phase: (A) 25mM KH₂PO₄, pH 3 (B) acetonitrile
 10% B to 40% B over 15 min
 Flow Rate: 1mL/min
 Pressure: <1030psi
 Temperature: 35°C
 Detection: UV, 260nm
 Injection: 5µL 25mM KH₂PO₄, pH 3 containing 100µg/mL each analyte



OFFER
 SEE OFFER PAGE 5

Separations of the five tetracyclines are illustrated in Figures B, C, and D. Minocycline and oxytetracycline will coelute from the C18 column, but are very well separated by the RP-AmideC16 and C8 columns (Figures C and D). The Discovery RP-AmideC16 column provides the best resolution of minocycline and oxytetracycline. The background around the chlortetracycline and doxycycline peaks is caused by impurities in the samples. Note that on-column quantities of analytes differed among Figures B, C, and D.

The ability of the RP-AmideC16 phase to separate minocycline and oxytetracycline might be explained by the hydrogen bonding between the amide functionality of the phase and the hydroxy functionality of oxytetracycline. Such differences in selectivity show the advantage of using the amide column for a difficult separation.

This study showed that mixtures of tetracycline antibiotics could be separated by reversed phase HPLC, using Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. Except for coelution of the tetracyclines minocycline and oxytetracycline on the Discovery C18 column, excellent resolution was achieved in every separation.

For additional information about this application
contact our Applications Laboratory at
aplab@sial.com

Ordering information

Phase	Prod. No.
Discovery Columns	
15cm x 4.6mm ID, 5µm particles	
Discovery C8	59353-U
Discovery C18	504955
Discovery RP-AmideC16	505013
Discovery Selectivity Packs¹	
5cm x 2.1mm ID Columns	55720-U21
15cm x 2.1mm ID Columns	55722-U21
5cm x 4.6mm ID Columns	55720-U
15cm x 4.6mm ID Columns	55722-U
25cm x 4.6mm ID Columns	55724-U
¹ Four columns of equal dimensions, one of each Discovery phase (C18, RP-AmideC16, C8, Cyano).	

References

- Levy, S.B., Multidrug Resistance-A Sign of the Times in The New England Journal of Medicine 338 (19), May 7, 1998.
- Levy, S.B., The Antibiotic Paradox: How Miracle Drugs Are Destroying the Miracle Plenum, New York, 1992.
- Steven, J.B., Chem. & Indus. News 2, 1996.
- Fedeniuk, R.W, P.J. Shand, J. Chromatogr. A 812: 3-15 (1998).
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2.1	7.5	569254-U
2.1	10	CUSTOM
2.1	15	569255-U
4.6	5	569250-U
4.6	7.5	569251-U
4.6	10	CUSTOM
4.6	15	569252-U
2.1	5	568500-U
2.1	10	568501-U
2.1	15	568502-U
2.1	25	568503-U
4	5	568510-U
4	10	568511-U
4	15	568512-U
4	25	568513-U
4.6	5	568520-U
4.6	10	568521-U
4.6	15	568522-U
4.6	25	568523-U
10	5	568530-U
10	10	568531-U
10	15	568532-U
10	25	568533-U
21.2	5	568540-U
21.2	10	568541-U
21.2	15	568542-U
21.2	25	568543-U
10	5	568630-U
10	10	568631-U
10	15	568632-U
10	25	568633-U
21.2	5	568640-U
21.2	10	568641-U
21.2	15	568642-U
21.2	25	568643-U

Discovery HS F5

2.1	3.3 (call to order)	567501-U
2.1	5	567500-U
2.1	10	567502-U
2.1	15	567503-U
3.0	3.3 (call to order)	567505-U
3.0	15 (call to order)	567542-U
4	5	567530-U
4	10	567531-U
4	15	567532-U
4.6	3.3 (call to order)	567509-U
4.6	5	567504-U
4.6	10	567506-U
4.6	15	567507-U
2.1	5	567508-U
2.1	10	567510-U
2.1	15	567511-U
2.1	25	567512-U
4	5	567533-U
4	10	567534-U
4	15	567535-U
4	25	567536-U
4.6	5	567513-U
4.6	10	567515-U
4.6	15	567516-U
4.6	25	567517-U
10	5	567518-U
10	10	567519-U
10	15	567537-U
10	25	567520-U
21.2	5	567521-U
21.2	10	567539-U
21.2	15	567522-U
21.2	25	567523-U
10	5	567524-U
10	10	567538-U
10	15	567525-U
10	25	567526-U
21.2	5	567527-U
21.2	10	567540-U
21.2	15	567528-U
21.2	25	567529-U

Discovery HS PEG

2.1	5	567400-U
2.1	10	567402-U
2.1	15	567403-U
4	5	567430-U
4	10	567431-U
4	15	567432-U
4.6	5	567404-U
4.6	10	567406-U
4.6	15	567407-U
2.1	5	567408-U
2.1	10	567410-U
2.1	15	567411-U
2.1	25	567412-U
4	5	567433-U
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4	15	567435-U
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4.6	5	567413-U
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10	15	567419-U
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21.2	15	567422-U
21.2	25	567423-U
10	5	567424-U
10	10	567438-U
10	15	567425-U
10	25	567426-U
21.2	5	567427-U
21.2	10	567440-U
21.2	15	567428-U
21.2	25	567429-U

HPLC ARTICLE

Discovery Solves HPLC Problems

PROBLEM 4: Poor Resolution of Closely-eluting Compounds

Demonstration 2: Solving co-elution of steroid compounds.

The steroidal compounds in this application are very similar in structure. A C18 column was not able to fully resolve several of the pairs. However, by using a functionalized reversed-phase column with enhanced polargroup selectivity, in this case a Discovery HS F5, resolution of all five compounds was achieved with a simple mobile phase.

Demonstration 3: Solving co-elution of prednisolone and an impurity.

Prednisolone is a naturally occurring steroid, chemically related to hydrocortisone. HPLC is often used to assay the purity of the synthetic form. A C18 column was not able to fully resolve a small impurity of prednisolone that appeared on the downslope of the main peak. However, by using a functionalized reversed-phase column with enhanced polargroup selectivity, in this case a Discovery HS F5, resolution of this compound was achieved.

Figure 1: Optimized Separation of Corticosteroids on Discovery HS F5

Column: Discovery HS F5, 5cm x 4.6mm ID, 5µm particles
 Mobile Phase: 60:40, Water:Methanol
 Flow Rate: 1.5mL/min
 Temperature: 60°C
 Detection: UV, 240nm
 Injection Volume: 5µL
 Sample: 10mg/mL mixture of corticosteroids in mobile phase

1. Hydrocortisone
2. Prednisolone
3. Prednisone
4. Corticosterone
5. Hydrocortisone acetate

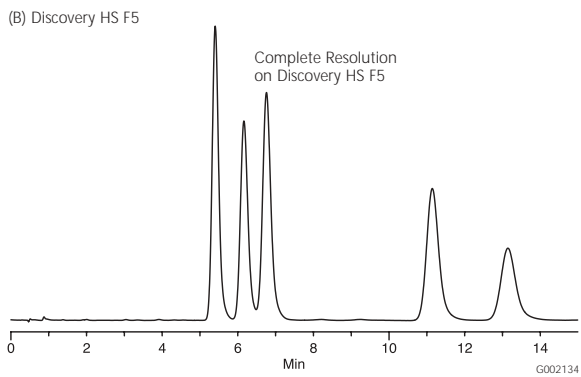
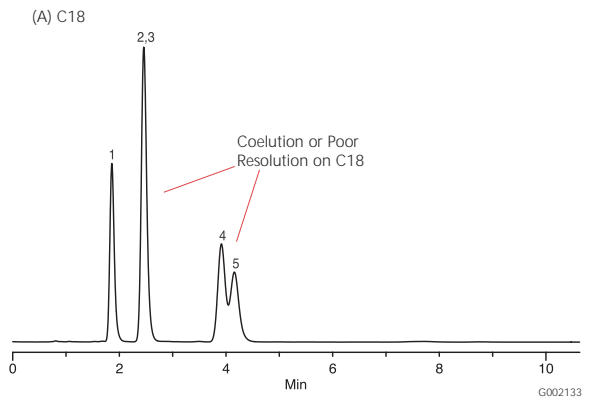
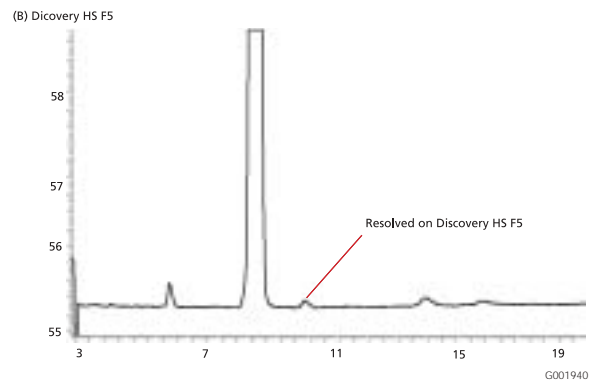
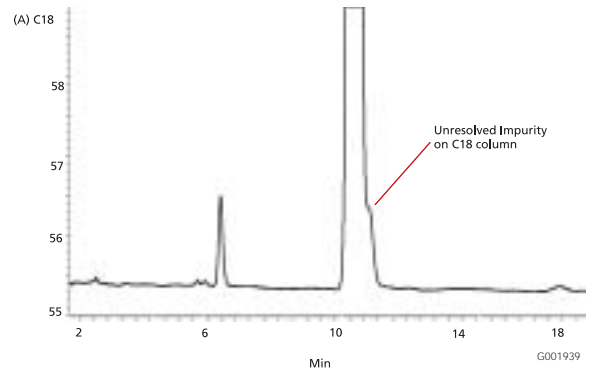


Figure 1: Discovery HS F5 Resolves Prednisolone and Impurity

Column: (A) C18 or (B) Discovery HS F5, 25cm x 4.6mm ID, 5µm particles
 Mobile Phase: (A) Water; (B) CH3CN; 0-26% B in 20 minutes
 Flow Rate: 1.5mL/min
 Det.: UV at 243nm
 Temp.: Ambient
 Inj.: 10µL, Prednisolone (0.25mg/mL)



**Demonstration 4:
Solving co-elution of Pepstatin A and impurity.**

Pepstatin A is a pentapeptide pepsin inhibitor, isolated from cell culture broths. In this example, note that the separation on a standard C18 column shows a small peak that is barely resolved from the large pepstatin A peak. When the same gradient was run on Discovery HS F5 column, baseline resolution of the smaller impurity peak was achieved. Changing from a C18 to a functionalized reversed-phase changed selectivity, allowing an impurity peak, previously unresolved, to be separated and detected.

**Demonstration 5:
Solving co-elution of hydroxylated flavone compounds.**

Flavones are a group of naturally-occurring, multi-ring, hydroxyl-containing compounds that are widely studied for their nutritional value and their use in preventive medicine. On a C18 column, co-elution of some flavone components typically occurs. By changing to a functionalized reversed-phase column, in this instance a Discovery HS PEG column, resolution as well as shorter run time were achieved.

Figure 1: Discovery HS F5 Resolves Impurity from Pepstatin A

Column: (A) C18 or (B) Discovery HS F5, 25cm x 4.6mm ID, 5µm particles
 Mobile Phase: 0.1% TFA in (A) Water; (B) 1:3, Water:CH3CN; 40 – 65% B in 30 minutes
 Flow Rate: 1.3mL/min
 Det.: UV at 215nm
 Temp.: Ambient
 Inj.: 20µL, Pepstatin A (1mg/mL in CH3OH containing 1% CH3OOH)

1. Hydrocortisone
2. Prednisolone
3. Prednisone
4. Corticosterone
5. Hydrocortisone acetate

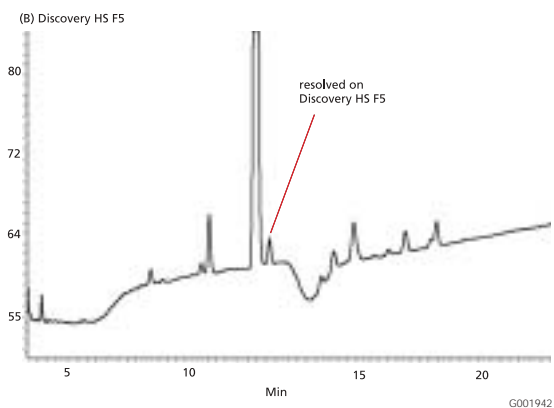
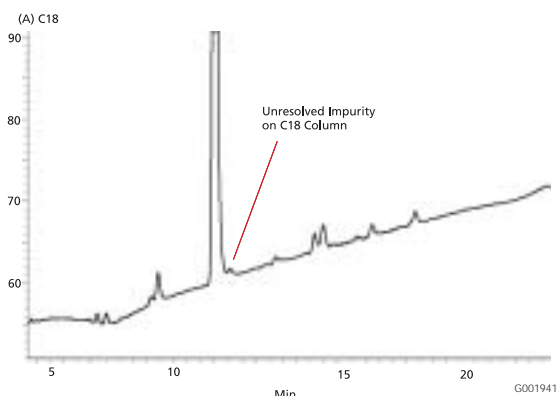
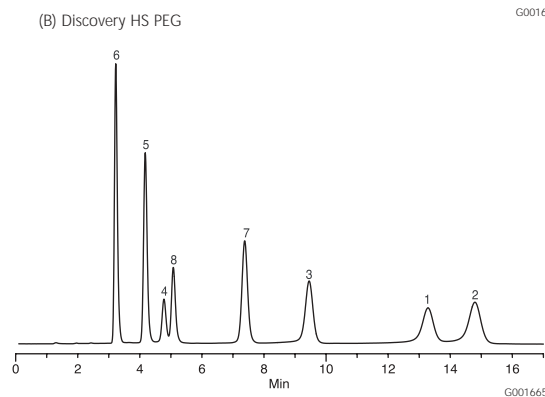
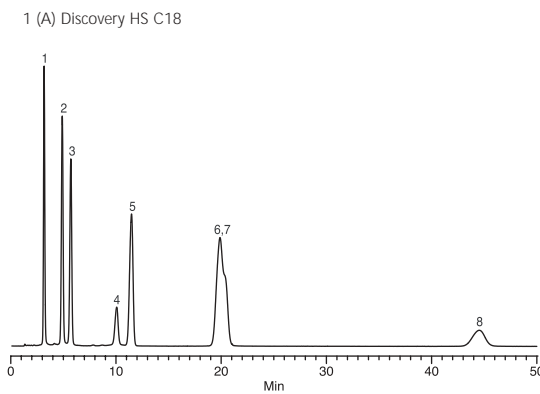


Figure 1: Flavones Demonstrate Dramatic Selectivity Differences Between HS PEG and C18

Column: 15cm x 4.6mm ID, 5µm particles
 Mobile Phase: 45:55 0.1% Formic Acid in Water : 0.1% Formic Acid in MeOH
 Flow Rate: 1.0mL/min
 Temp.: 30°C
 Det.: UV at 254nm
 Inj.: 10µL

Sample: 50µg/mL of each

1. Myricetin
2. Quercetin
3. Luteolin
4. Baicalein
5. 7-Hydroxyflavone
6. Flavone
7. Chrysin
8. 5-Hydroxyflavone



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0.02	6000	Orange	Z227293
0.03	5000	Green	Z226955
0.055	1000	Black	54994
1/8" OD			
0.062	5000	Natural	54995

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58764	Plug, 1/16" (nut required)
58766	Cap, 1/16" (nut and ferrule required)

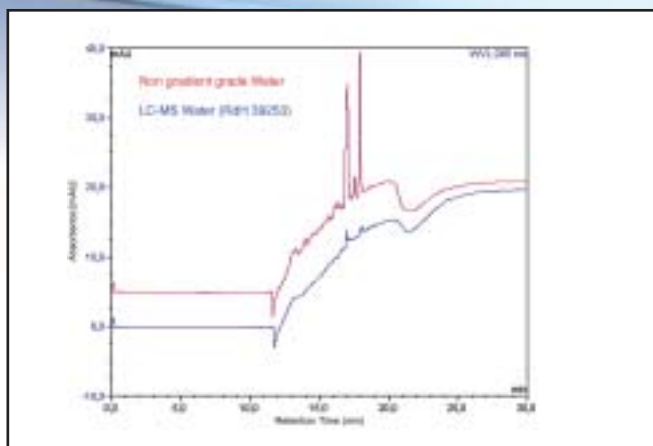
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34668	Acetonitrile with 0.1% formic acid	2.5 L
34675	Water with 0.1% acetic acid	2.5 L
34678	Acetonitrile with 0.1% acetic acid	2.5 L
34672	Methanol with 0.1% acetic acid	2.5 L
34674	Water with 0.1% ammonium acetate	2.5 L
34669	Acetonitrile with 0.1% ammonium acetate	2.5 L
34670	Methanol with 0.1% ammonium acetate LC-MS CHROMASOLV®	2.5 L

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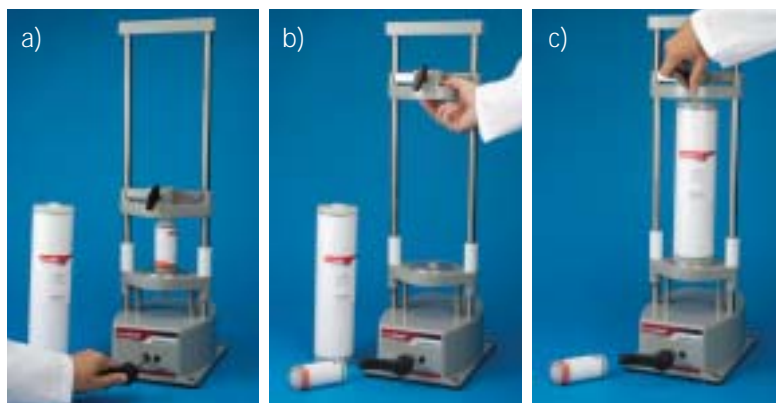
- Pre-compressed cartridges eliminate the expense of additional compression barrels
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Improve Performance:

- Spherical particles result in low band spreading
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- Unobstructed cartridges allow monitoring of the separation

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- Remove the small used cartridge by turning the handle to idle position and pulling it out.
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SPE ARTICLE

Determination of α -/ β Thujone and Related Terpenes in Absinthe using Solid Phase Extraction and Gas Chromatography**Chromatography in Food Science**

The power of GC/MS to resolve and identify structurally similar compounds, combined with efficient sample prep and high purity reference standards provide analytical chemists with powerful tools to analyze the complex natural product samples. The study of active ingredients in a controversial alcoholic beverage, absinthe, described in this report is an example of such an application.

Absinthe: "La fee verte"

Absinthe is a green, anise-flavored bitter spirit with high alcohol content. Many absinthe recipes exist and all contain essential oil extracts of various herbs including hyssop, fennel, and anise, but the most important component is *Artemisia absinthium* (wormwood). Absinthe became an extremely popular beverage throughout all levels of society in Europe and America during the 19th century. Widespread abuse of absinthe caused deleterious health effects and as a result it was banned in most countries. Although still illegal in the US, absinthe was recently reintroduced to the European market. This reintroduction along with the improvement in analytical techniques prompted the development of new analytical methods to measure the active ingredients in absinthe in order to understand and control its potentially toxic effects. Terpenes are one group of compounds found in absinthe that have been targeted as toxic agents.

Absinthe terpenes: α -/ β -thujone, anethole, and others

Some of the terpenes *Artemisia absinthium* contributes to absinthe are α -/ β -thujone, anethole, fenchone and linalool (Figure 1). Thujone, especially the α -isomer, has been blamed for the adverse health effects of absinthe. The EU has set a maximum concentration of 35 mg/L for thujone in bitter spirits. Thujone levels < 2 mg/L indicate the absence of *Artemisia absinthium* in the recipe. Meeting the analytical challenges of thujone

The identification and quantitation of thujone and related terpenes in absinthe present formidable analytical challenges from several standpoints. First, naturally occurring thujone exists in α and β isomeric forms in a roughly 1:2 ratio. While they differ only in the stereochemistry of the 4-methyl group, the α -isomer is more toxic than the β -isomer. Early analytical methods were unable to detect low levels of thujone, nor resolve the α - and β -isomers. Modern GC solved this problem. Because the stereochemistry affects internal hydrogen bonding, the two isomers have different boiling points, allowing their separation by capillary GC. Second, high purity standards were difficult to obtain, but are now commercially available from Fluka. Third, the thujone-containing *Artemisia absinthium* extract also contains other terpenes and plant-derived compounds that interfere with thujone identification and quantitation. Early methods employed liquid-liquid extraction or distillation to isolate the thujone-containing fraction. These methods were complicated, time consuming, inefficient and gave low recovery. Modern solid phase extraction (SPE) methods provide rapid, efficient isolations and high recovery from complicated matrices. Additionally, mass spectroscopy (MS) now provides analysts with a tool for structural determination unavailable and likely unimaginable to their 19th

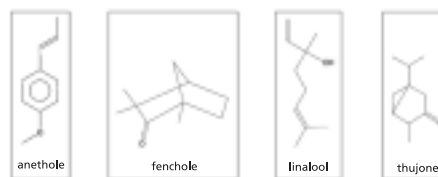
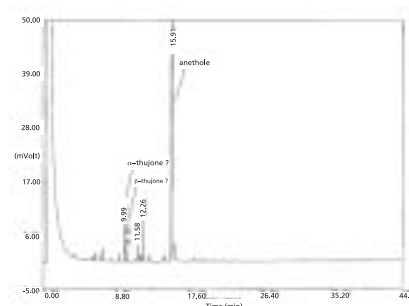
century colleagues.

Case study: Absinthe samples

We sought to apply these high purity reference standards and improved analytical techniques to the problem of α - and β -thujone analysis in absinthe. A complete description of the method along with detailed background on absinthe chemistry appears in reference 1.

Sample prep using SPE

Lipophilic terpenes were isolated from polar compounds in various commercial absinthe samples using 1 mL SPE-tubes packed with C18-silica particles (Supelco Discovery DSC-18) following a simple procedure. The SPE tube was activated with 1 mL methanol, followed by conditioning with 1 mL water. Exactly 1 mL of the absinthe sample (spiked or unspiked) was applied to the tube. Polar compounds were removed by washing with 1 mL water to dryness. Terpenes were eluted from the tube with 1 mL methanol directly into the sample vial.

Figure 1 Structures of some terpenes found in absinthe**Fig. 2** GC/FID analysis of Candela absinthe sample suggesting high levels of α - and β -thujone.

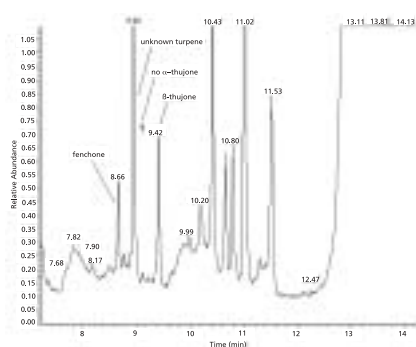
An additional benefit of this SPE method is identified which absinthe samples contained artificial vs. natural colorings. Artificial colors were eluted with steps 3 and 4 (sample addition and washing), whereas natural colors (presumably from the lipophilic chlorophyll) remained with the terpene fraction, but did not interfere with terpene quantitation.

Recovery of standard anethole was between 95–100%. Quantitation in absinthe samples was done with external calibration using pure standards. The recovery of α -thujone varied between 40 and 70% depending on the matrix of the different absinthes. Therefore, the amount of α -thujone was calculated with standard addition to each sample. Level of β -thujone, where no high purity standard is currently available, was calculated using the response factor of α -thujone in the same sample. With this procedure, a detection limit of 0.1

Ordering Information

Absinthe sample	α -Thujone (mg/L)	β Thujone (mg/L)	Anethole (mg/L)
Mata Hari	1.9	9.9	11 (0)
Grüne Fee	2.4	9.0	7 (6)
Versinthe Blanche	24.7 (5.2)	4.8	3220
La Fee 68°	0.0	0.0	1184
Pernod 68°	0.0	0.0	1056
Francois Guy	4.8	20.0	1334
Emile Pernot 68°	3.2	0.9	412
Oxygenee	2.2	0.0	1025
Segarra	0.0	0.0	1208
Candela	33.3 (0.0)	7.9	2669
Rote Fee Anis	19.2 (0.0)	0.0	740
Absente	2.6	1.6	1365
Prohibido	10.3 (0.0)	3.0	337
Martini rosso	0.0	14.4 (0.0)	0
Fuchs Absinth	8.7 (0.0)	0.0	434
Pernod Anis	0.0	0.0	2484
Pernod Tarragona	0.8	0.5	n.d.

Fig. 3 GC/MS (TIC) analysis of Candela absinthe sample showing no α -thujone, but high levels of an unknown compound.



mg/L and a precision of 5% were achieved. For anethole, the precision was better than 2%, but with much higher absolute values.

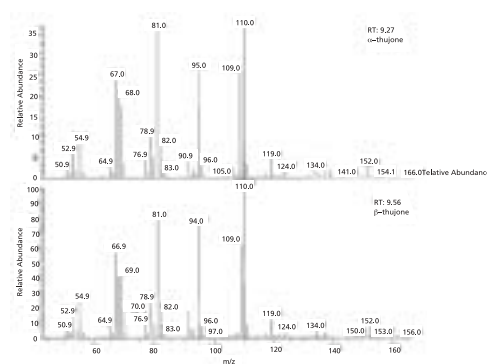
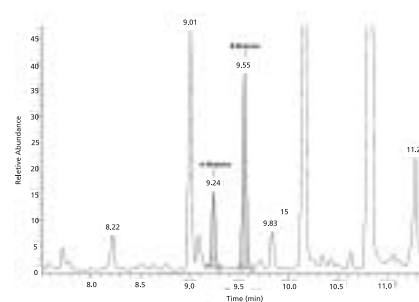
Analysis by GC/FID and GC/MS

Quantitation of terpenes in the SPE extract was done by GC/FID using a 30m x 0.25 mm ID, 5% diphenyl-, 95% dimethylpolysiloxane capillary column, 0.25 μ m film thickness. The temperature program was 50–250°C (5°/min) with He carrier gas. 1 μ L samples were injected with a split ratio of 1:20 on a split/splitless injector. GC/MS was used for structural identification. The column, split ratio, injection volume, carrier gas and flow, as well as the temperature program were identical to the GC/FID conditions. MS conditions: full scan mode 50–500 d, total ion current (TIC) to obtain full scan spectra for subsequent library search.

Results: Thujone levels of absinthe samples

The α - and β -thujone and anethole levels measured using the SPE-GC/FID and GC/MS methods described above in seventeen commercially available absinthe samples appear in Table 1. A GC/FID chromatogram of Candela brand absinthe appears in Figure 2. Note that if only GC/FID was used, this sample would appear to have erroneously high levels of α -thujone because of the elution of an unknown compound close to the α -thujone peak. Further analysis of this sample using GC/MS (Figure 3) confirmed the presence of the unknown compound, but no peaks eluting in the retention window where α -thujone elutes. In order to identify this unknown peak, an Artemisia absinthium sample was extracted analyzed by GC/MS (Figure 4). Comparison of structural libraries ultimately identified this compound as linalool.

Fig. 4a GC/MS (TIC) analysis of Artemisia absinthium extract. The 9.01 minute peak was identified as linalool.



Conclusion

Sample prep using SPE followed by GC/FID or GC/MS analysis provide a fast and reliable technique to isolate, identify and quantify absinthe terpenes, especially α -thujone. The SPE method has significant advantages in speed, recovery and accuracy compared to distillation and liquid/liquid extraction. In addition, this method provides simultaneous determination of several terpenes, and avoided interference of α -thujone by linalool. The use of MS for qualification of the quantified peaks is highly recommended. Our results also suggest that historically high results for α -thujone reported in absinthes may have been caused by analytical methods that were not able to differentiate between the above-discussed terpene species. Having access to high purity reference standards is important for proper peak identification and quantification using standard addition.

References

- (1) Emmert, J., G. Sartor, F. Sporer and J. Gummersbach: Deutsche Lebensmittel-Rundschau 100, 9, 352-356 (2004).

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- Change sample matrix (i.e., solvent exchange) to improve compatibility with your analytical method

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Dimension.	Pack	DSC-18 Prod. No.	DSC-18 Lt Prod. No.	DSC-8 Prod. No.	DSC-Ph Prod. No.	DSC-CN Prod. No.	DSC-Si Prod. No.	DSC-Diol Prod. No.	DSC-NH ₂ Prod. No.	DSC-SAX Prod. No.	DSC-WCX Prod. No.	DSC-SCX Prod. No.
Discovery SPE Tubes												
50mg/1mL	108/pk	52601-U	52610-U	52703-U	52723-U	52693-U	52652-U	52747-U	52635-U	52661-U	52737-U	52684-U
100mg/1mL	108/pk	52602-U	52611-U	52707-U	52725-U	52694-U	52653-U	52748-U	52636-U	52662-U	52739-U	52685-U
500mg/3mL	54/pk	52603-U	52613-U	52713-U	52727-U	52695-U	52654-U	52751-U	52637-U	52664-U	52741-U	52686-U
500mg/6mL	30/pk	52604-U	52615-U	52714-U	52728-U	52696-U	52655-U	52752-U	52638-U	52665-U	52742-U	52688-U
1g/6mL	30/pk	52606-U	52616-U	52716-U	52731-U	52697-U	52656-U	52753-U	52640-U	52666-U	52743-U	52689-U
2g/12mL	30/pk	52607-U	52618-U	52717-U	Custom	52698-U	52657-U	Custom	52641-U	52677-U Kit only	52744-U	52690-U
5g/20mL	20/pk	52608-U	52621-U	52718-U	Custom	52699-U	52658-U	Custom	52642-U	52688-U	52745-U	52691-U
10g/60mL	20/pk	52609-U	52622-U	52722-U	Custom	52700-U	52659-U	Custom	52644-U	52699-U	52746-U	52692-U
Bulk packing	100g	52600-U	52623-U	52723-U	52727-U	52722-U	52651-U	52729-U	52712-U	52714-U	52728-U	52721-U
Discovery SPE-96 Well Plates												
100mg/well	1ea	575603-U	575606-U	575627-U	575630-U	575624-U	575609-U	575636-U	575615-U	575618-U	575633-U	575621-U
50mg/well	1ea	575602-U	575605-U	575628-U	575631-U	575625-U	575608-U	575637-U	575616-U	575619-U	575634-U	575622-U
25mg/well	1ea	575601-U	575604-U	575629-U	575632-U	575626-U	575607-U	575638-U	575617-U	575620-U	575635-U	575623-U
Discovery Büchner Funnels												
55mmID x 30mmH, 12.5g												
	6 qty/pk	Custom	Custom	Custom	Custom	Custom	52591-U	Custom	Custom	Custom	Custom	Custom
70mmID x 40mmH, 25g												
	6 qty/pk	Custom	Custom	Custom	Custom	Custom	52592-U	Custom	Custom	Custom	Custom	Custom
90mmID x 48mmH, 50g												
	6 qty/pk	Custom	Custom	Custom	Custom	Custom	52593-U	Custom	Custom	Custom	Custom	Custom
110mmID x 66mmH, 100g												
	6 qty/pk	Custom	Custom	Custom	Custom	Custom	52594-U	Custom	Custom	Custom	Custom	Custom

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33031-U	25ml	BSA + TMCS + TMSI (3:2:3)	Sylon BTZ	++	++	++	++	+
33155-U	25ml	BSTFA + TMCS (99:1)	Sylon BFT	+++	+++	+++	+++	+
33046	20 x 1ml	HMDS + TMCS (3:1)	Sylon HT	++	++	++	++	+
394610-25ml	25ml	Reacta-Sil Concentrate	HMDS:TMCS (2:1)	++	++	++	++	+
33039	25ml	HMDS + TMCS + Pyridine (3:1:9)	Sylon HTP	++	++	++	++	+
394882-5ml	5ml	MTBSTFA	-	+++	++	+++	++	++
375934-5ml	5ml	MTBSTFA + TBDMCS (99:1)	-	++	++	++	++	++
33156-U	25ml	TMSI + Pyridine (1:4)	Sylon TP	+++	++	-	++	+
33092-U	10 x 1ml	T-Butyldimethylsilylimidazole-dimethylformamide	-	+	+	-	+	-
15222	1ml, 5ml, 25ml	N,O-Bis(trimethylsilyl)acetamide	BSA	++	++	++	++	+
15243	100ml, 25ml	N,O-Bis(trimethylsilyl)trifluoroacetamide	BSTFA	++	++	++	++	+
52619	10ml, 50ml, 250ml	Hexamethyldisilazane	HMDS	+	++	+	++	+
69479	1ml, 5ml, 25ml	N-Trimethylsilyl-N-methyltrifluoroacetamide	MSTFA	++	++	++	++	+
69478	1ml, 5ml	MSTFA + 1 % TMCS	MSTFA/TMCS					
50992	5ml, 25ml	MSTFA + Ethanethiol Ammoniumiodide	MSTFA activated I					
44156	5ml, 25ml	MSTFA + Trimethylsilyl + Ethanethiol	MSTFA					
12124	5ml, 25ml	MSTFA + Imidazole	MSTFA					

i Information Request.....1407

GC ARTICLE

The Analysis of Alcoholic Beverages on the 30m x 0.25mm ID, 1.0µm SPB-20 Capillary Column

Traditional methods for monitoring aroma components in alcoholic beverages have employed packed column GC in fusel oil analyses. In this application, we used a 30m x 0.25mm ID, 1.0µm SPB-20 capillary column to separate 12 common components regularly monitored in alcoholic beverages.

In addition to ethanol and water, alcoholic beverages contain a variety of compounds that are produced during fermentation and/or aging. These compounds impart many of the flavor and aroma characteristics familiar in certain beverages. To ensure consistency in finished product quality and flavor, many distilleries monitor the presence and relative levels of these compounds. Isoamyl alcohol, for example, is an aroma component in rum. At very low levels, this compound has a fruity, pleasant odor. At high levels, the aroma of isoamyl alcohol is unpleasant. Its separation from active amyl alcohol is considered critical if monitoring because these two compounds are normally produced together. Compounds such as ethyl acetate, 1-propanol, isobutyl alcohol, and isoamyl alcohol are monitored as part of quality control in many beverages. Collectively, these compounds are referred to as fusel oils.

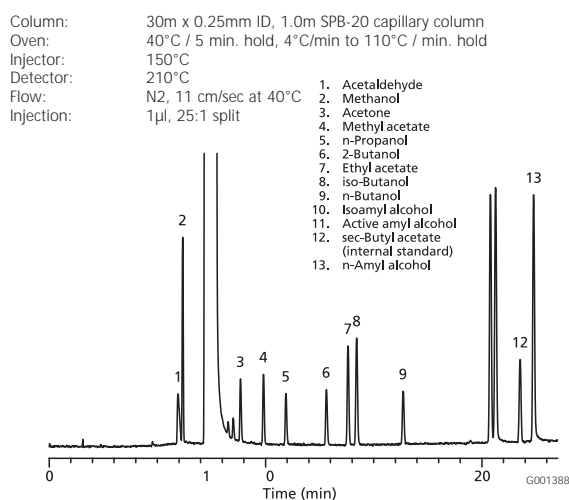
Traditional methods have employed packed column GC in fusel oil analysis. In this application, we used a 30m x 0.25mm ID, 1.0mm SPB-20 capillary column to separate 12 common components regularly monitored in alcoholic beverages. We also assayed several real world beverage samples.

Figure A illustrates the separation of the monitored compounds in a matrix of 40% ethanol in water. The ethanol matrix did not interfere, and all components were separated. The aroma compounds, isoamyl and active amyl alcohol, were separated almost to baseline. The inertness of the SPB-20 resulted in good peak shape for all compounds, including the alcohols. Many locations worldwide testing for these compounds have difficulty acquiring low cost helium. For this reason, we chose nitrogen as the carrier to show that it can be used with good results. If helium is used with the analysis, one can expect a decrease in the analysis time.

Figure B illustrates the use of the SPB-20 for the analysis of a variety of alcoholic beverage samples. This data shows that the SPB-20 capillary column is an excellent choice and a viable alternative to the use of packed columns for alcoholic beverage analysis.



Figure A. Alcoholic Beverage Analysis on the 30m x 0.25mm ID, 1.0µm SPB-20



Description	Cat. No.
SPB-20 Fused Silica Capillary Column	
30m x 0.25mm ID x 1.0µm film	24196
Fused silica columns manufactured under HP US Pat. No. 4,293,415.	
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24086	30	0.2
24087-U	60	0.25
24196-U	30	1.0
0.32mm ID FUSED SILICA		
24088	30	0.25
24194-U	60	1.0
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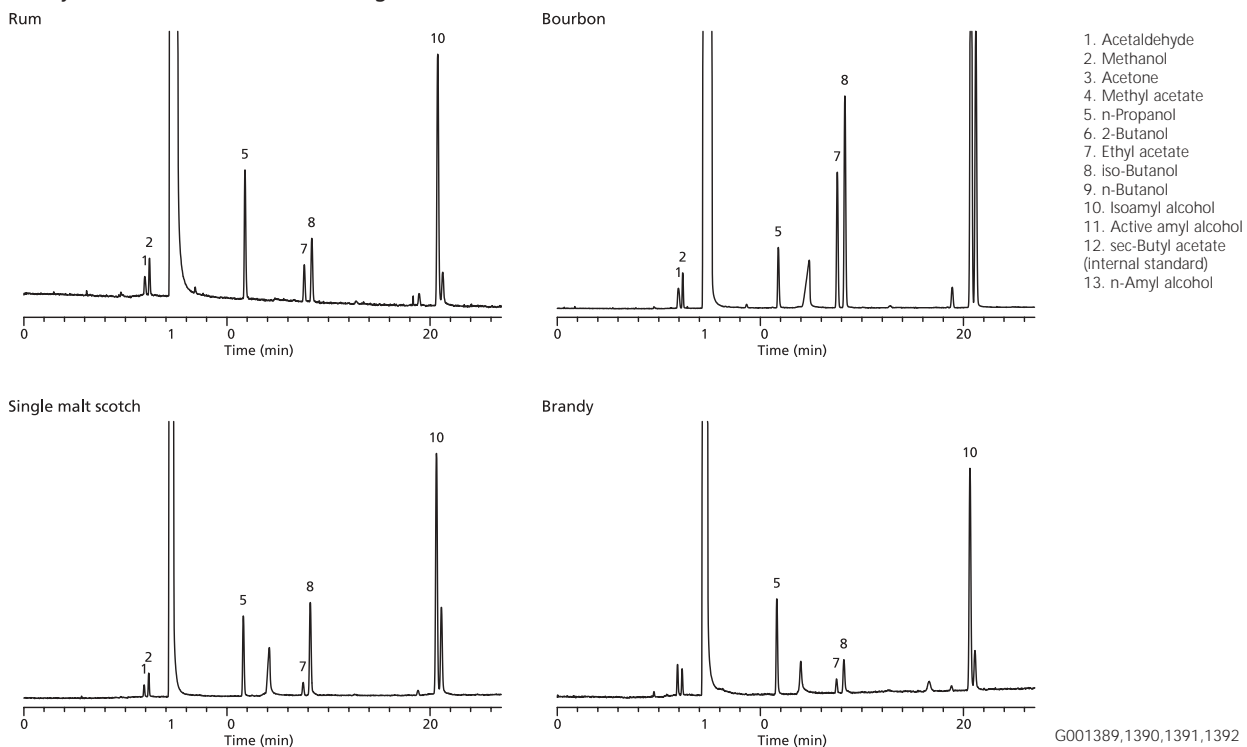
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Figure B: Analysis of Various Alcoholic Beverages on the SPB-20



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Modified Microliter					
1.2µL	7701.2	26p	51mm	#1	28617-U
Microliter Syringe, Fixed Needle					
5µL	75N	26s	51mm	#1	28613-U
10µL	701N	26s	51mm	#2	28614-U
10µL	701N	26s	51mm	#1	28615-U
Gastight Syringe, Fixed Needle					
25µL	1702N	26s	51mm	#1	28649-U
100µL	1710N	26s	51mm	#1	28651-U
250µL	1725N	26s	51mm	#1	28652-U
500µL	1750N	26s	51mm	#1	28653-U

GC SAMPLING: A200S, PAL INSTRUMENTS

Gastight Syringe

1mL	1000LTN	23	56mm	#5	28621-U
1mL	1001LTN	26	56mm	#5	28622-U
2.5mL	1002LTN	22	56mm	#5	28626-U
2.5mL	1002LTN	26	56mm	#5	28627-U
5mL	1005LTN	23	56mm	#5	28628-U
5mL	1005LTN	26	56mm	#5	28629-U

HPLC SAMPLING: HTS PAL INSTRUMENTS

Microliter Syringe, Fixed Needle

10µL	701N	22s	51mm	#3	28618-U
Microliter Syringe, Fixed Needle					
10µL	1701N	22s	51mm	#3	28632-U
25µL	1702N	22s	51mm	#3	28633-U
100µL	1710N	22s	51mm	#3	28634-U
100µL	1710N	22	51mm	#3	28635-U
250µL	1725N	22	51mm	#3	28636-U
1mL	1001LTN	22	56mm	#3	28637-U
2.5mL	1002LTN	22	56mm	#3	28638-U
5mL	1005LTN	22	56mm	#3	28639-U

Replacement Plungers

MODEL	DESCRIPTION	CAT. NO.
1701CTC	for 10µL CTC Syringe	28641-U
1702CTC	for 25µL CTC Syringe	28642-U
1710CTC	for 100µL CTC Syringe	28643-U
1725CTC	for 250µL CTC Syringe	28644-U
1750CTC	for 500µL CTC Syringe	28645-U
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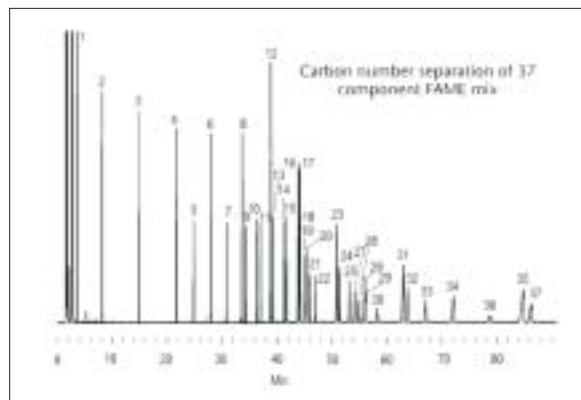
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Similar structures and boiling points among airborne solvents monitored in the workplace often require two GC columns that offer differing selectivities to separate and identify the solvents collected. The highly polar SUPELCOWAX 10 capillary column resolves many industrial solvents based on polarity. In contrast, the nonpolar PTE-5 capillary column resolves solvents by boiling point. The shifts in retention times resulting from the complementary separation mechanisms of the two columns can aid analysts in identifying and quantifying solvents in complex mixtures.

OSHA regulations require the monitoring of solvent concentrations in industrial workplace atmospheres to minimize health risks to workers. The OSHA-specified procedures normally use adsorbent sampling tubes to collect and concentrate the analytes. The analytes are solvent-desorbed from the tube and analyzed using gas chromatography. Similar structures and boiling points among

Table 1. Elution Order Comparison

1. Hexane	11. Methanol (64.5°C)*
U Unknown	3. Acetone (56°C)
2. 1,1-Dichloroethylene	14. Isopropanol (82°C)
3. Acetone	2. 1,1-Dichloroethylene (32°C)
4. Methyl acetate	4. Methyl acetate (54°C)
5. trans-1,2-Dichloroethylene	15. Methylene chloride (40°C)
6. Tetrahydrofuran	5. trans-1,2-Dichloroethylene (48°C)
7. Carbon tetrachloride	U Unknown
8. 1,1,1-Trichloroethane	9. 1,1-Dichloroethane (57°C)
9. 1,1-Dichloroethane	13. Methyl ethyl ketone (79.6°C)
10. Ethyl acetate	1. n-Hexane (69°C)
11. Methanol	23. 2-Butanol (99.5°C)
12. Isopropyl acetate	10. Ethyl acetate (77°C)
13. Methyl ethyl ketone	21. Chloroform (61°C)
14. Isopropanol	29. Isobutanol (107°C)
15. Methylene chloride	6. Tetrahydrofuran (66°C)
16. Benzene	34. Methyl Cellosolve® (124.5°C)
17. Propyl acetate	8. 1,1,1-Trichloroethane (74°C)
18. Trichloroethylene	26. 1,2-Dichloroethane (84°C)
19. Methyl isobutyl ketone	16. Benzene (80°C)
20. Isobutyl acetate	33. 1-Butanol (118°C)
21. Chloroform	12. Isopropyl acetate (89°C)
22. Tetrachloroethylene	7. Carbon tetrachloride (77°C)
23. 2-Butanol	18. Trichloroethylene (87°C)
24. Toluene	25. 1,4-Dioxane (101°C)
25. 1,4-Dioxane	39. Cellosolve (136°C)
26. 1,2-Dichloroethane	17. Propyl acetate (102°C)
27. n-Butyl acetate	37. Isoamyl alcohol (113°C)
28. 2-Hexanone (MBK)	19. Methyl isobutyl ketone (116°C)
29. Isobutanol	24. Toluene (111°C)
30. Isoamyl acetate	20. Isobutyl acetate (117°C)
31. p-Xylene	42. N,N-Dimethylformamide (153°C)
32. m-Xylene	28. 2-Hexanone (MBK) (127 °C)
33. 1-Butanol	22. Tetrachloroethylene (121°C)
34. Methyl Cellosolve	27. n-Butyl acetate (126°C)
35. Amyl acetate	38. Chlorobenzene (132°C)
36. o-Xylene	31. m-Xylene (139°C)
37. Isoamyl alcohol	32. p-Xylene (139°C)
38. Chlorobenzene	30. Isoamyl acetate (142°C)
39. Cellosolve	43. Cyclohexanol (161°C)
40. Styrene	40. Styrene (145°C)
41. Cyclohexanone	41. Cyclohexanone (157°C)
42. N,N-Dimethylformamide	36. o-Xylene (144°C)
43. Cyclohexanol	44. Butyl Cellosolve (171°C)
44. Butyl Cellosolve	35. Amyl acetate (148°C)
45. 2-Methylcyclohexanol	47. 1,1,2,2-Tetrachloroethane (147°C)
46. 1,2-Dichlorobenzene	45. 2-Methylcyclohexanol (165°C)
47. 1,1,2,2-Tetrachloroethane	46. 1,2-Dichlorobenzene (172°C)
48. o-Cresol	48. o-Cresol (191°C)
49. p-Cresol	49. p-Cresol (202°C)
50. m-Cresol	50. m-Cresol (203°C)

* Boiling points

Figure B: Industrial Solvents on a SUPELCOWAX 10 Column

Column: SUPELCOWAX 10, 30m x 0.53mm ID, 1.0µm film
 Cat. No.: 25301-U
 Oven: 40°C (5 min) to 200°C at 5°C/min
 Carrier: helium, 5mL/min
 Det.: FID
 Inj.: 1µL mixed solvents, split 50:1

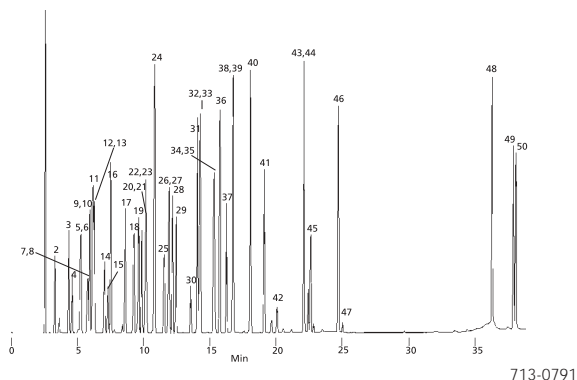
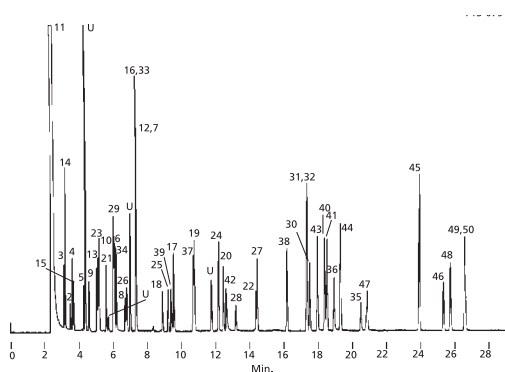


Figure B: Industrial Solvents on a SUPELCOWAX 10 Column

Column: PTE-5, 30m x 0.32mm ID, 1.0µm film
 Cat. No.: 24159
 Oven: 40°C (5 min), to 130°C at 4°C/min
 Carrier: helium, 20cm/sec (set at 135°C)
 Det.: FID
 Inj.: 1µL mixed solvents, split 100:1



713-0790

the solvents often require two GC columns that offer differing separation mechanisms or selectivities to separate and identify the solvents collected.

Some analysts split the sample injection to the two columns to allow simultaneous detection and analysis with two detectors. This technique can be used when a client of an environmental lab requests a complete analysis of all possible solvents present on the adsorbent tube. Other analysts use one column, confirming the analysis on a second column only when coelutions are suspected, based on the known solvent use at an industrial site.

SUPELCOWAX 10 and PTE-5 capillary columns provide complementary separations of solvent mixtures. The SUPELCOWAX 10 capillary column offers a highly polar, bonded phase that resolves many industrial solvents based on polarity (Figure A). The PTE- capillary column has a nonpolar,

poly(5% diphenyl/95% dimethylsiloxane) phase that resolves solvents approximately by boiling point (Figure B, Table 1). If you know the boiling point of a compound, you can use the chromatogram to estimate its retention time on a PTE-5 column. (Some polar compounds may elute out of boiling point order due to their interaction with the phase.)

The SUPELCOWAX 10 column provides greater retention of extremely polar compounds (e.g., alcohols) compared to the PTE- column, as shown by the methanol shift (peak 11). Polar compounds, which can tail or be poorly resolved on other phases, are better resolved and display better peak symmetry on the SUPELCOWAX 10 column. The SUPELCOWAX 10 column also provides baseline separation of the o-, p-, and m-xylenes, and nearly baseline separation of the p- and m-cresols. We recommend the combined use of the PTE-5 and SUPELCOWAX 10 columns for the most reliable identification and quantitation of solvents in complex mixtures.

sigma-aldrich.com/supelcowax10

Ordering information

Description	Cat. No.
SUPELLOWAX 10 Fused Silica Capillary Column	
30m x 0.53mm ID, 1.0µm film	25301-U
PTE-5 Fused Silica Capillary Column	
30m x 0.32mm ID, 1.0µm film	24159

See the Supelco catalog for other column dimensions.
Fused silica columns manufactured under HP US Pat. No. 4,293,415.

Information Request.....1410

OMI Indicating Gas Purifiers

OMI Indicating Purifier Removes as Much Oxygen as Most Nonindicating Purifiers

- Simultaneously remove O₂, water vapor, CO, CO₂, most sulfur compounds, most halogen compounds, alcohols, phenols to less than 10ppb
- Purify helium, hydrogen, nitrogen, argon-methane
- Color change indicates purifier exhaustion
- Glass body does not diffuse air or off-gas
- Ideal for Hall, ECD, GC/MS detection systems
- OMI-4 purifier protects multiple instruments (three times the capacity of OMI-2 tubes)

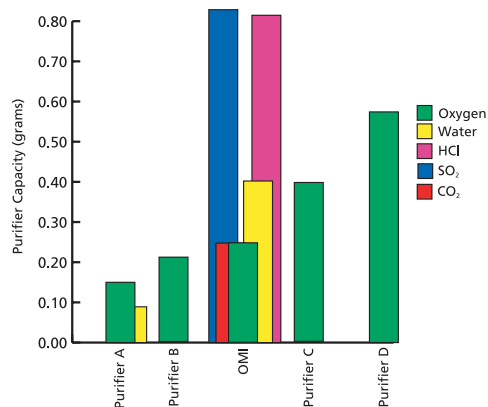


Irreversibly remove contaminants from carrier gas.

Install an OMI purifier downstream from your primary gas purifying device, and tell at a glance whether or not oxygen and water vapor are being effectively eliminated from your system. The OMI purifier will provide point-of-use gas polishing and final visual assurance of gas quality before the gas enters the GC. OMI purifier tubes contain Nanochem resin, developed for the demanding gas purity needs of the semiconductor manufacturing industry. As little as 1ppm of oxygen or water will change the indicating resin from black to brown. Spent tubes are easily replaced. Simply unscrew the end assembly from the tube holder and replace the tube. The design prevents air from entering the new tube during installation.

Note: For optimum performance, we do not recommend storing OMI tubes for longer than 6 months.

OMI Indicating Purifier Removes as Much Oxygen as Most Nonindicating Purifiers



Protect Your Column from Many Carrier Gas Contaminants

CONTAMINANT	OMI PURIFIER	INDICATING DEVICES	NON-INDICATING OXYGEN TRAPS
Oxygen	Yes	Yes ¹	Yes ¹
Water	Yes	No	Maybe
Carbon monoxide	Yes	No	Maybe
Carbon dioxide	Yes	No	No
Alcohols/Phenols	Yes	No	No
Sulfur-containing compounds	Yes	No	No ²
Halogen-containing compounds	Yes	No	No ²

¹If incoming oxygen level does not exceed 10ppm.

²Corrosive compounds may poison some of these devices.

SUPELCOWAX

10

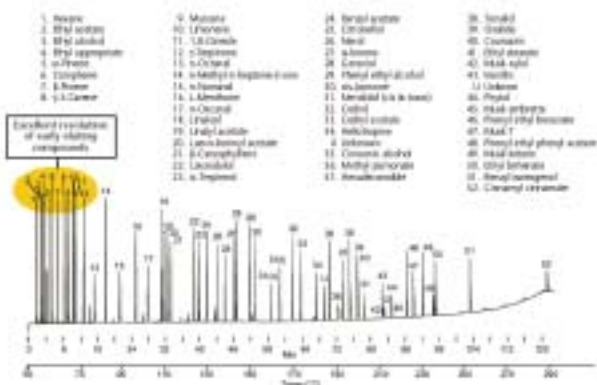
The *hottest* polar GC column on the market

SUPELCOWAX 10 is the most famous polar column for high temperature applications on the market.

It is the only column with a temperature limit from 35°C up to 280°C.

Use SUPELCOWAX 10 for separation and purity analysis of polar compounds like alcohols, aromatics and other solvents, flavors, fragrance and FAMES.

i Information Request.....1411



Ordering Information

Description	(µm)	BETA	CAT. NO.
0.10mm ID FUSED SILICA			
5	0.10	250	25025-U
10	0.10	250	25026-U
15	0.10	250	24343
0.20mm ID FUSED SILICA			
30	0.20	250	24169
60	0.20	250	24170
0.25mm ID FUSED SILICA15			
15	0.25	250	24077
30	0.25	250	24079
60	0.25	250	24081
30	0.50	125	24284
0.32mm ID FUSED SILICA			
15	0.25	320	24078
30	0.25	320	28080-U
60	0.25	320	24082
15	0.50	160	24083
30	0.50	160	24084
60	0.50	160	24085-U
30	1.0	80	24211
60	1.0	80	24212
0.53mm ID FUSED SILICA			
15	0.50	265	25324
30	0.50	265	25325
60	0.50	265	25385
15	1.0	133	25300-U
30	1.0	133	25301-U
60	1.0	133	25391
30	2.0	63	25375-U
60	2.0	63	25376

TRIAL OFFER 10% OFF

on the listed SUPELCOWAX 10 GC columns.

Quote promotion code T12 to qualify for this offer.
Offer valid until 31. December 2004

Do you know

Our NEW Liners FocusLiner™

- All liners deactivated with high temperature deactivation
- Innovative designs
- Optimal reproducibility
- Unrivalled precision, accuracy and reliability
- Simple to use
- Maximum sensitivity / detection levels
- Guaranteed to fit
- Guaranteed premium inertness

The FocusLiner™ is the first liner in the world to secure quartz wool by means of a tapering above and below the wool. This ensures the wool remains in the correct position to wipe the needle tip during injection. Reproducibility is greatly improved with chromatographers reporting Relative Standard Deviations (RSD's) of between 0.3 and 0.7%.

The new Fast FocusLiner™ has a narrow ID (<2.5mm) making it ideal to be used with Fast Capillary Columns (0.1mm ID).

Table of Liners:

Description	Dimension		Qty.	Cat No
Agilent for HP5890, HP6890, HP6850 & HP4890 Split/Splitless FocusLiner Single Taper, packed with quartz wool	78.5 x 6.3 x 4mm		1 5 25	2879901-U2879905-U 2879925-U
Split/Splitless FocusLiner, packed with quartz wool	78.5 x 6.3 x 4mm		1 5 25	2879801-U 2879805-U 2879825-U
Split/Splitless FAST FocusLiner, packed with quartz wool	78.5 x 6.3 x 2.3mm		1 5 25	2879601-U 2879605-U 2879625-U
Split/Splitless FAST FocusLiner, Single taper, packed with quartz wool	78.5 x 6.3 x 2.3mm		1 5	2879501-U 2879505-U 2879525-U
PerkinElmer Split/Splitless FocusLiner for AutoSystem and Clarus™ 500, packed with quartz wool	92 x 6.2 x 4.0mm		1 5	2879201-U 2879205-U
Split/Splitless FocusLiner for AutoSystem and Clarus 500, Single Taper, packed with quartz wool	92 x 6.2 x 4.0mm		1 5	2879101-U 2879105-U
Split/Splitless FocusLiner for PSS Injector and AutoSystem XL, packed with quartz wool	86.2 x 4.0 x 2mm		1 5	2878901-U 2878905-U
Varian for 1078 & 1079 Split/Splitless FocusLiner, Single Taper, packed with quartz wool	54 x 5 x 3.4mm		1 5	2875701-U 2875705-U
Split/Splitless FocusLiner, Dual Taper, packed with quartz wool	54 x 5 x 3.4mm		1 5	2875501-U 2875505-U
for 1075 & 1077 Split FocusLiner, packed with quartz wool	72 x 6.3 x 4mm		1 5	2875401-U 2875405-U
Split FocusLiner, tapered, packed with quartz wool	72 x 6.3 x 4mm		1 5	2874801-U 2874805-U
Split FAST FocusLiner, tapered, packed with quartz wool	72 x 6.3 x 2.3mm		1 5	2874701-U 2874705-U
Split FocusLiner, with top-end restriction, packed with quartz wool	72 x 6.3 x 4mm		1 5	2874901-U 2874905-U
Splitless FocusLiner, with top-end restriction, packed with quartz wool	72 x 6.3 x 4mm		1 5	2874601-U 2874605-U

CERTIFIED POLYMER STANDARDS FOR GEL PERMEATION CHROMATOGRAPHY....

Fluka now offers a new dimension of polymer analysis: the first certified polymer standards. Polystyrene (broad and narrow), PEO and polylactic acid standards are available.

By Rainer Walz....rwalz@sial.com

Knowledge of the molecular structure of polymers, including chemical composition, branching, average chain length and chain length distribution, is critical to the manufacture of many commercial products, from plastic bottles to canopies of fighter jets. One analytical difficulty facing polymer scientists was how to obtain polymer standards having a known chain length to calibrate the GPC analysis.

Because of the widespread need for certified polymer standards, and the impact of polymer quality on consumer safety, a government project was initiated. The polymer standards certifying process was coordinated by the German National Institute for Material Science and tested in Berlin by the Federal Institute for Materials Research and Testing (BAM). The certified parameters were carried out by well established round robin experiments done by several independent certified laboratories. In these experiments, independent characterization methods were employed by the laboratories under identical conditions. The data provided by the participating laboratories was statistically analyzed and validated.

Researchers at Fluka set out to provide polymer analysts with reliable, certified polymer standards that meet the rigorous requirements of the BAM tests. These standards are characterized using a battery of analytical tests, including GPC, GPC with light scattering detection (on-line and off-line) and GPC-viscometry (on-line and off-line). To further characterize our polymer standards, we also use MALDI-TOF, NMR, DSC and rheological testing when appropriate.

The data provided by this extensive Quality Assurance testing appears in the Certificate of Analysis for each lot of Fluka polymer standards. Some critical results presented in the Certificate of Analysis include the average molecular weight (Mw and Mn), polydispersity (D) and other information about the dimension of polymer solutions like intrinsic viscosity $\{\eta\}$.

Fluka Certified Polymer Standards can be used for calibration of the following analytical instruments:

- GPC
- Light scattering
- Viscosimetry
- Rheology
- MALDI-TOF
- Spectroscopy
- Surface-Analysis

They offer the benefits of:

- Well established and standardized characterization using different, independent analytical methods
- Round robin experiments that guarantee reliable parameters like Mw and Mn
- High accuracy
- Extensive quality documentation

The Certified Polymer Standards are supplied in 100 mg quantities, in glass vessels packed in aluminium bags to protect them from air and moisture. Each sample is accompanied with a Certificate of Analysis presenting all relevant test parameters. The lot-dependent average molecular weight is printed on the label.

Certified Polymer Standards

Cat. No	Brand	Polymer	Mw in D	Mw/Mn	Pack Size
44101	Fluka	Polyethylenoxide	10 160	1.01	100 mg
51876	Fluka	Polystyrene	87 600	1.08	100 mg
53397	Fluka	Polystyrene broad	205 600	2.26	100 mg
53395	Fluka	Polystyrene broad	349 800	2.25	100 mg
50243	Fluka	Polylactic Acid	77 450	1.68	100 mg
78464	Fluka	Polylactic Acid	225 200	1.98	100 mg



NEW PRODUCTS FOR NITROFURAN ANALYSIS

Sigma-Aldrich introduces seven new, derivatized and isotope-labelled standards for nitrofuran analysis.

By Rainer Walz....rwalz@sial.com

In 2002 nitrofuran antibiotic residues were found in fish products, including shrimp and catfish, originating in South East Asian countries. Later investigation revealed residues of the same compounds in poultry, duck and rabbit imported from this region.

Since the discovery of the occurrence of residues of nitrofurans and their metabolites (AOZ (3-amino-2-oxazolidinone), AMOZ (5-methylmorpholino-3-amino-2-oxazolidinone), AHD (1-Aminohydantoin hydrochloride) and SEM (semicarbazide hydrochloride), numerous scientists contacted Sigma-Aldrich asking for analytical standards. To meet their requests, we introduced the following four standards in 2003:

Ordering information

Cat. No	Brand	Name	Sinonym	Pack Size
33347	Riedel de Haën	3-Amino-2-oxazolidinone	AOZ	50mg
33349	Riedel de Haën	5-Methylmorpholino-3-amino-2-oxazolidinone	AMOZ	50mg
33870	Riedel de Haën	1-Aminohydantoin hydrochloride	AHD	10mg
33656	Riedel de Haën	Semicarbazide hydrochloride	SEM	100mg

Soon thereafter, we were asked to provide the derivatized and isotope-labelled standards as well. In response, we developed and introduced seven additional nitrofuran standards. Removing the need for the analyst to derivatize and label the compounds permits a significant reduction in analysis time. The 2-nitrobenzaldehyde derivative of nitrofuran is the most commonly used standard in nitrofuran analysis.

Ordering information

Cat. No	Brand	Name	Sinonym	Pack Size
33869	Riedel de Haën	5-(Morpholinomethyl)-3-(2-nitrobenzylidenamino)-2-oxazolidinone	2-NP-AMOZ	10mg
33868	Riedel de Haën	3-(2-Nitrobenzylidenamino)-2-oxazolidinone	2-NP-AOZ	10mg
33871	Riedel de Haën	(2-Nitrobenzaldehyde semicarbazone	2-NP-SCA	10mg
33870	Riedel de Haën	3-(2-Nitrobenzylidenamino)-2,4-imidazolidinedione	2-NP-AHD	10mg

For scientists performing nitrofuran analysis by LC-MS, we introduced three unique isotope marked standards:

Ordering information

Cat. No	Brand	Name	Sinonym	Pack Size
33881	Riedel de Haën	2-Nitrobenzaldehyde semicarbazone	AMOZ-D5	10mg
33880	Riedel de Haën	4,4,5,5-Tetradeutero-3-amino-oxazolidin-2-one	AOZ-D4	10mg
33882	Riedel de Haën	Semicarbazide-13C-15N2hydrochloride	SCA-HCl (13C, 15N)	10mg

The analysis of nitrofuran drug residue is based on the detection of the tissue-bound metabolites of the nitrofuran parent compound. There are no immunochemical or microbiological screening methods presently available. Most commonly the analysis is carried out by LC-MS or LC-MS/MS techniques.

"Determination of the metabolites of nitrofuran antibiotics in animals tissues by HPLC-tandem mass spectrometry", A. Leitner, P. Zvllner, W. Lindner, J. Chromatogr. A, 939 (2001) 49-58.

"Analysis of protein-bound metabolites of furazolidone and furaltadone in pig liver by high performance liquid chromatography and liquid chromatography-mass spectrometry.", E. Horne, A. Cadogan, M. O'Keeffe and L.A.P. Hoogenboom, The Analyst, 121 (1996) 1463-1468.

"The use of pig hepatocytes to study the nature of protein-bound metabolites of furazolidone; a new analytical method for their detection.", L.A.P. Hoogenboom, M. van Kammen, M.C.J. Berghmans, J.H. Koeman and H.A. Kuiper, Food and Chemical Toxicology, 29 (1991), 321-328.



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