

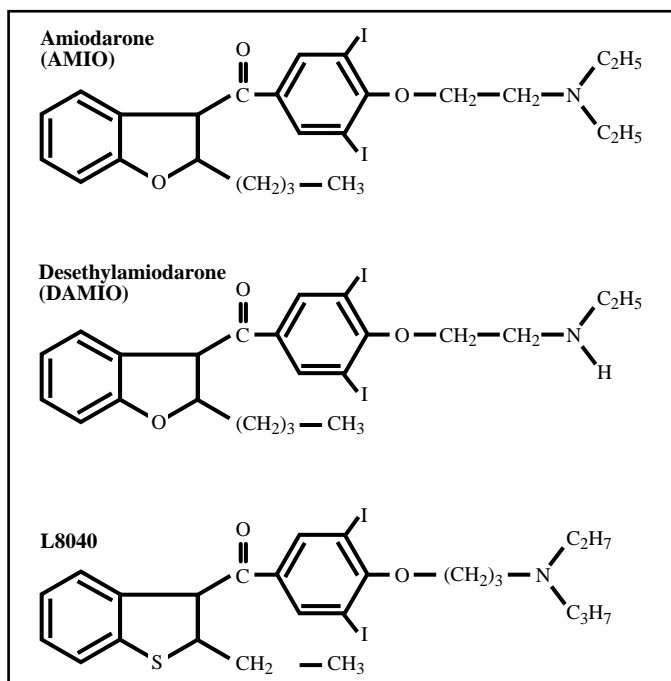
3M

Empore™ High Performance Extraction Disk Cartridges

Method Summary

Amiodarone from Serum**C8-HD 4mm/1mL****Summary**

An analytical method is described for the solid phase extraction (SPE) of the antiarrhythmic drug amiodarone and its metabolite, desethylamiodarone, from 250 μ L serum using C8-HD Empore™ Extraction Disk Cartridges (4mm/1mL). This particular method highlights use of the SPE membrane with a centrifuge. Method optimization details are included and a comparison was made with conventional packed columns (100mg/1mL) to demonstrate the advantages of the membrane format for solid phase extraction.



Overall, the extraction of amiodarone and its metabolite from serum using particle-loaded membranes achieves excellent linearity, recovery and precision. Improved concentrating ability and reduced solvent volume requirements are demonstrated, compared with traditional packed columns. In addition, elution of drugs from the membrane sorbent was efficiently accomplished using a small volume of HPLC mobile phase, eliminating analyte instability problems and the need for additional time-consuming evaporation and reconstitution steps.

Introduction

Membranes with chemically bonded chromatographic particles enmeshed in polytetrafluoroethylene (PTFE) microfibrils are new analytical tools for improved solid phase extraction of drugs. The effectiveness of the thin PTFE membrane loaded with C8 bonded silica (8 μ m avg.) is demonstrated for the extraction of the antiarrhythmic drugs amiodarone and its metabolite, desethylamiodarone, from serum. These drugs are often monitored to minimize toxic side effects seen at concentrations > 2.5 μ g/mL.

Solid phase extraction and HPLC analysis were performed and the following parameters were used to assess performance: linearity, recovery, precision, elution volume requirements, and lowest limit of quantitation. A comparative evaluation with conventional large particle (40 μ m avg.) sorbents packed in columns demonstrates the advantages of the membrane format for solid phase extractions.

Materials

Reagents, Standards and Supplies

Acetic Acid

1 M, HPLC Grade

Internal Standard Solution

L8040, 10 µg/mL in methanol
Store at room temperature

Wash Solution

Acetonitrile/water (20/80, v/v)

Eluting Solution/HPLC Mobile Phase

Water/acetonitrile/tetrahydrofuran/acetic acid/n-butylamine (100/900/20/0.3/0.4, by volume)

Standard Solution

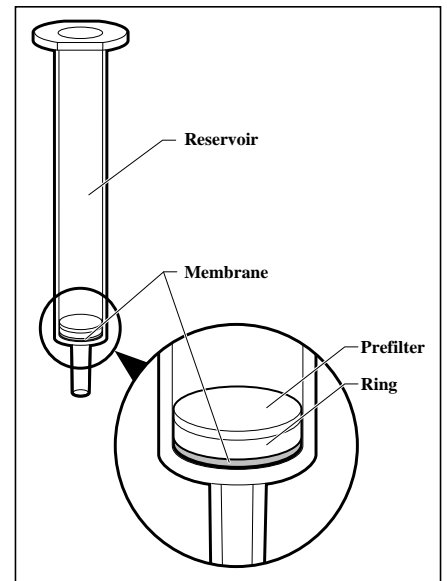
Stock solution contained 300 µg/mL each of amiodarone and desethylamiodarone in methanol. Working standard solutions were prepared by diluting stock solution with drug-free serum. Store in 1 mL air-tight vials in freezer (-20°C).

Empore Extraction Disk Cartridge

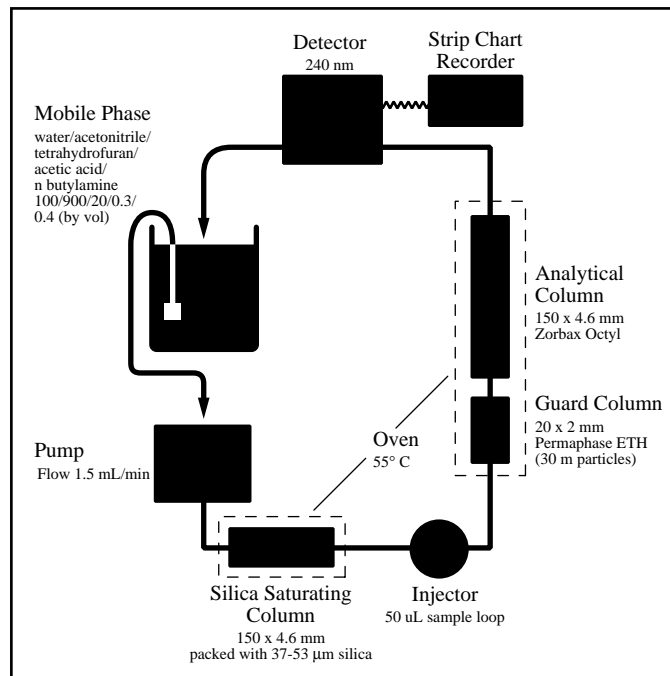
4mm/1mL containing octyl (C8) bonded silica, high density formulation (C8-HD)

HPLC Conditions

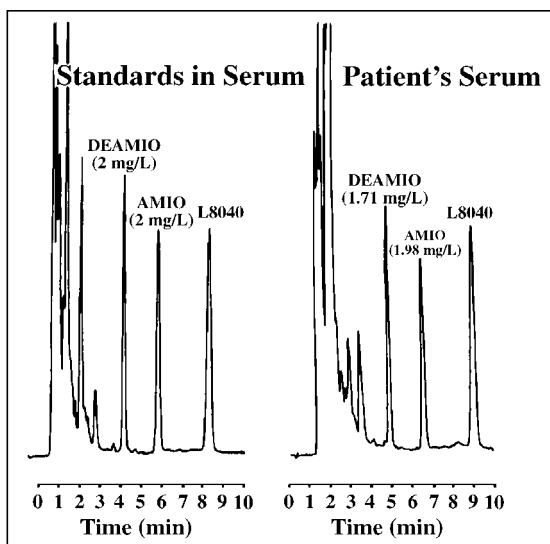
See diagram below. Note: use of silica saturating column is important to minimize deterioration of analytical column.



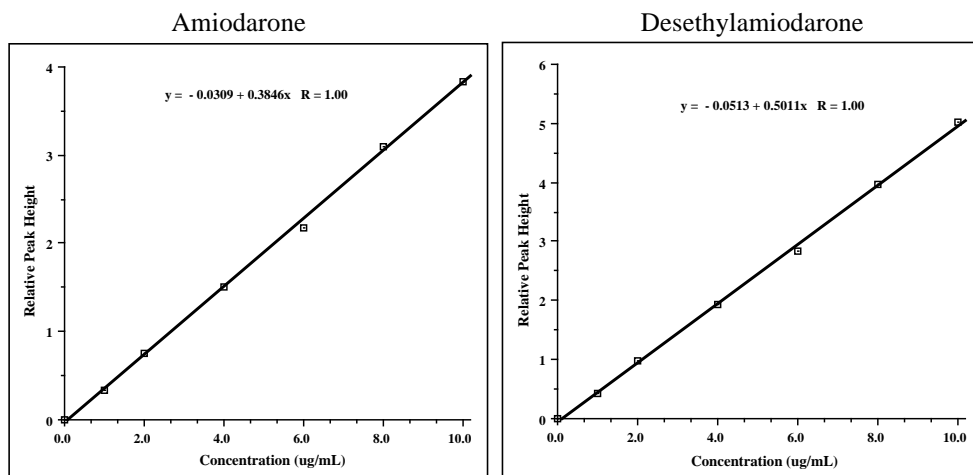
High Performance Liquid Chromatography (HPLC)



Typical Chromatograms



Linearity



Precision and Recovery

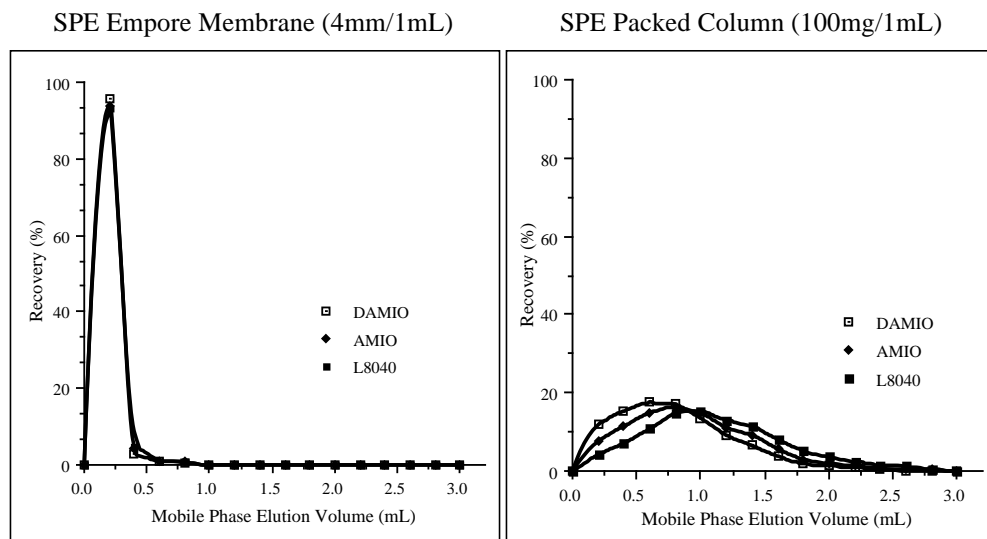
	Precision (between-run, n = 15)			Mean μg/mL	SD μg/mL	CV %	Recovery (at 300 μg/mL)	Sensitivity (lowest limit of quantitation)
	Mean μg/mL	SD μg/mL	CV %					
AMIO	0.415	0.015	3.7	3.06	0.094	3.1	92-95%	0.05 μg/mL
DAMIO	0.412	0.013	3.3	3.06	0.096	3.2	90-93%	0.05 μg/mL

SD = standard deviation
CV = coefficient of variation

Comparative Study with Packed SPE Columns

The drug extraction was duplicated on conventional SPE columns with loosely packed large particle (40 μm) C8 bonded silica. Both membrane and column contained C8 sorbent from the same manufacturer and differed only in particle size. The most remarkable findings were the volume of solvent required for complete elution of drugs (0.75 mL membrane; 2.75 mL column) and resulting differences in analytical sensitivity when the eluate is directly injected onto the HPLC system.

Elution Volume Profiles

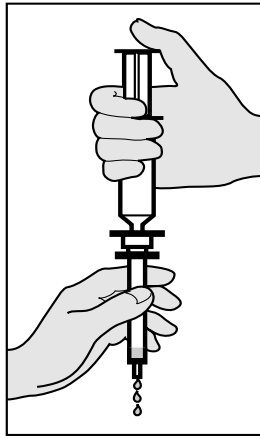


Performance Data

	Empore Membrane	Packed Column
Physical Characteristics		
Bonded Silica	C8	C8
Particle Size	8 μm	40 μm
Effective Weight	4 mg	100 mg
Extraction Performance		
Precision (overall CVs)		
AMIO	3.4%	3.5%
DAMIO	3.2%	3.4%
Linearity	up to at least 10 $\mu\text{g/mL}$	up to at least 10 $\mu\text{g/mL}$
Lowest Limit of Quantitation	0.05 $\mu\text{g/mL}$	0.18 $\mu\text{g/mL}$
Volume of Elution	0.75 mL	2.75 mL
Clarity of Eluate	Clear	Particles present*
Total Solvent Volume for Extraction	2.2 mL	4.4 mL

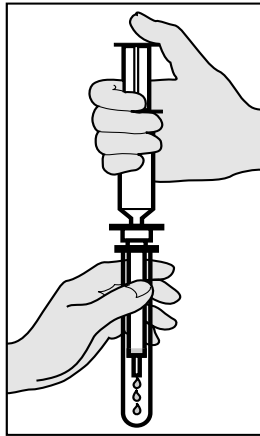
*Centrifugation of eluate required to prevent plugging of HPLC

Extraction Procedure



1. Combine into a test tube and mix:
 - A. 250 μL serum (or calibrators)
 - B. 800 μL 1N acetic acid
 - C. 100 μL methanol with internal standard (L8040)
2. Prime the membrane by manually pushing through 3 drops of methanol followed by 3 drops of water.
3. Load sample mixture into cartridge and force through membrane by either:
 - A. centrifuging 100-120 $\times g$ for 5 min OR
 - B. manually applying pressure with a syringe OR
 - C. pulling with a vacuum

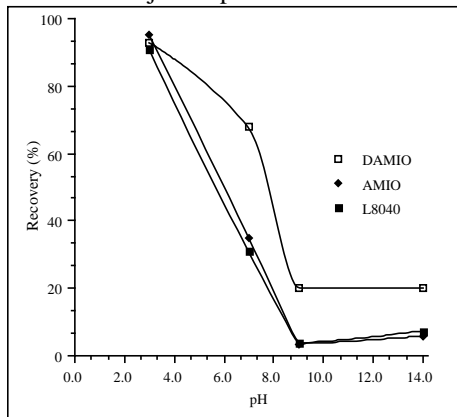
With centrifugation, consistent flow rates can be maintained and many samples can be processed simultaneously.



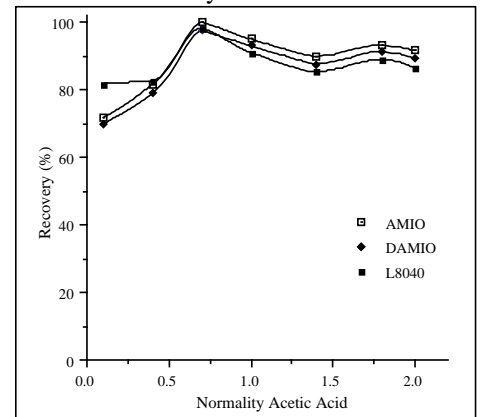
4. Manually push through 500 μL of acetonitrile/-water, 20/80 (v/v) to remove retained proteins and interferences.
5. Elute the retained drugs by pushing through 800 μL of mobile phase (same as for HPLC) and collect in polypropylene tube.
6. Inject 50 μL of eluate onto HPLC column.
7. Relative retention time and relative peak height techniques are used for identification and quantitation, respectively.

Optimization Charts

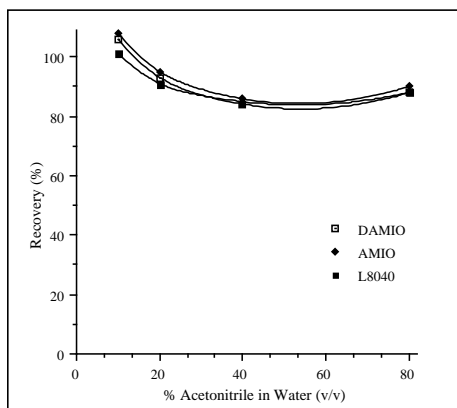
Adjusted pH of Serum



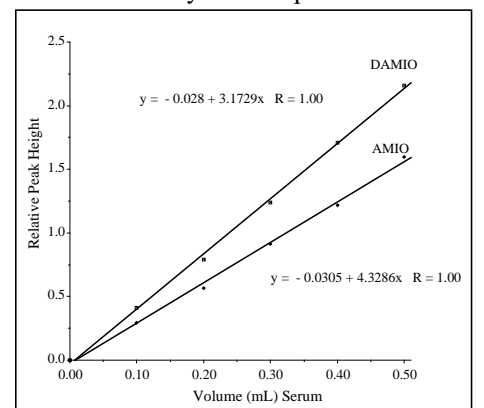
Normality of Acetic Acid



Acetonitrile Content of Wash Solution



Relationship of Serum Sample Volume to Analytical Response



Conclusions

The C8 particle-loaded Empore Extraction Disk Cartridge (4mm/1mL) efficiently extracts amiodarone and its metabolite from serum.

Method performance data demonstrate:

- Between-run precision (%CV) of 3.1 - 3.7%
- Extraction recoveries of 90 - 95%
- Linearity from 0.05 µg/mL (limit of quantitation) to at least 10 µg/mL

In some respects, both membrane and traditional large particle packed columns performed similarly in this assay. However, distinct advantages were observed for the membrane. Remarkably, the drugs could be eluted from the membrane with HPLC mobile phase and the eluate directly injected onto the HPLC system, without additional concentration steps or loss of sensitivity.

When compared with SPE columns, the Empore Disk Cartridge:

- Allowed for a smaller elution volume
- Afforded 3.5 times greater analytical sensitivity
- Used only half as much reagent solvent volume
- Produced essentially particle-free eluates

The improved concentrating ability of the Empore Disk Cartridge can be attributed to the robust configuration of the sorbent within an inert support. The small 8 µm (avg.) C8 bonded silica particles tightly enmeshed within PTFE fibrils give greater surface area and increased capacity per milligram of sorbent than conventional large 40 µm (avg.) particle sorbents loosely packed in columns.

Reference

G.L. Lensmeyer, D.A. Wiebe and T. Doran, "Application of the Empore Solid Phase Extraction Membrane to the Isolation of Drugs from Blood. I. Amiodarone and Desethylamiodarone," *Therapeutic Drug Monitoring* **13**, 244-250 (1991).

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