Quick analyses of antiarrhythmic drugs are necessary for clinical laboratories to process large numbers of samples. By using the 5cm SUPELCOSIL LC-8-DB column, with only two stock mobile phase solutions, clinicians can sequentially analyze five classes of antiarrhythmic drugs and achieve accurate chromatography.

Key Words:
- antiarrhythmic drugs
- HPLC
- SUPELCOSIL HPLC columns
- solid phase extraction

Many times, physicians prescribe drugs that are effective in small doses at low blood levels and have relatively high toxicity. The blood level of one or more active metabolites must often be determined simultaneously with the administered drug. This makes imperative the need for accurate, rapid drug monitoring procedures. Since clinical laboratories are processing large numbers of samples and a widening diversity of compounds, they can make efficient use of time and staff by:
- selecting columns suitable for a variety of analyses
- using short columns to reduce analysis time
- minimizing variation of the mobile phase composition to reduce equilibration time when changing analyses

As an example of analyses that fit these criteria, a clinician can sequentially analyze five classes of antiarrhythmic drugs on a 5cm x 4.6mm SUPELCOSIL™ LC-8-DB column. An isocratic separation of each antiarrhythmic drug, related metabolites, and internal standards can be completed in three to five minutes (Figure A). Mobile phases in these analyses consisted of acetonitrile and 0.02M phosphate buffer, in various proportions. Since the mobile phases are quite similar, only two stock solutions are needed, with addition of triethylamine for analysis A1. Equilibration is rapid from one analysis to another.

Therapeutic levels of procainamide, lidocaine, quinidine, and disopyramide all fall in the mg/L range. Since the quantities of drugs shown in Figures A1-A4 correspond to quantities expected in 0.004 to 0.08mL of blood plasma, the sensitivity of these analyses is more than adequate. Because the therapeutic range for propranolol is only 50-150µg/L, fluorescence detection has been recommended for this drug. However, the quantity of drug we detected by UV in Figure A5 would be expected in 0.13 to 0.40mL of serum. UV detection, then, although less sensitive than fluorescence, can be a viable alternative when proper clean-up procedures are used with the sample.

◊ Common contaminant in quinidine, usually equal to about 5-10% of the quinidine peak height.
Under the conditions used in Figure A3, the active metabolite 3-hydroxyquinidine (when present) will elute before quinidine. Samples containing propranolol (Figure A5) may have to be digested with enzymes (beta-glycuronidase/arylsulfatase) prior to analysis, because 75% of the drug and 95% of the active metabolites are present in conjugated forms (2). Solutions of 4-hydroxypropranolol apparently are unstable, unless an antioxidant (2% sodium metabisulfite) is added (3).

Where gradient capabilities and multiple wavelength detection permit, an analysis like that shown in Figure B can be set up. All five classes of antiarrhythmic drugs, related active metabolites, and permit, an analysis like that shown in Figure B can be set up. All five classes of antiarrhythmic drugs, related active metabolites, and internal standards can be analyzed in 12 minutes without changing the mobile phase (Figure B). Reequilibration takes 10 minutes, so samples can be injected at 22 minute intervals.

Figure B. Separation of Antiarrhythmic Drugs by Gradient Elution

| Column: SUPELCOSIL LC-8-DB (58344), 5 cm x 4.6mm, 5um packing, with Supelguard guard column (59553), 2cm x 4.6mm, 5um packing |
| Mobile Phase: acetonicitrate: 0.02M H3PO4 (pH to 3.0 with KOH), 0.02% TEA (pH 3.3), gradient, equilibrate at 3% ACN, then to 30% ACN over 10 min. and hold 3 min. |
| Temp.: 35°C |
| Flow Rate: 2.0mL/min. |
| Det.: UV at 254nm, 0.02 AUFS |
| Sample: 10µL, on-column amounts indicated on figure |

1. Procainamide (35ng) 9. 4-Hydroxy propranolol (0.25µg) Solid phase extractions (4, 5) have been suggested for several of these drugs. These procedures produce clean samples and are most suitable for rapid analyses on short columns. We also recommend using a disposable, cartridge-type 2cm x 4.6mm guard column in front of the 5cm analytical column. The guard column should be filled with the same 5 micron packing, rather than a pellicular packing. All separations shown here were performed using such a guard column.

Five separate but related analyses are available for rapidly separating antiarrhythmic drugs. These analyses employ a 5cm x 4.6mm SUPELCOSIL LC-8-DB column, the interrelated mobile phases described above, and different wavelengths of UV for detection. The shorter analytical columns, when compared to 15 or 25cm columns, offer a substantial cost advantage.

Ordering Information:

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPELCOSIL LC-8-DB* Column 5cm x 4.6mm, 5um packing</td>
<td>58344</td>
</tr>
<tr>
<td>Supelguard LC-8-DB Guard Column Kit, includes a 2cm column filled with a 5um packing, plus column holder and connecting hardware</td>
<td>59553</td>
</tr>
<tr>
<td>2cm replacement Supelguard LC-8-DB guard columns, pk. of 2</td>
<td>59563</td>
</tr>
</tbody>
</table>

For solid phase extraction columns, see our Chromatography Supplies Catalog.

References
4. A typical procedure for preparing procainamide containing samples involves washing 100µL of serum with 25% aqueous methanol and t-butyl ether, then eluting the sample from a solid phase C18 extraction column, using 0.1N HCl in methanol. The material is dried, then reconstituted in mobile phase.
5. In a typical procedure for preparing disopyramide containing samples, the sample is eluted from a solid phase silica extraction column into a small volume of 0.1N HCl, using butylic chloride/isopropanol (90:10). The aqueous phase is dried, then reconstituted in mobile phase. References not available from Supelco.

Acknowledgment
Thanks to Jerry Thoma of South Bend Medical Research Foundation for suggestions regarding solid phase extractions.

Trademarks

SUPELCOSIL, Supelguard – Sigma-Aldrich Co.