the particular advantages that "dirty" extracts may be easily processed by repeated "washing" and that the stationary phase is disposable. In our experience, however, the main drawback to the technique has been the difficulty of transferring an appreciable proportion of drug in a dried extract to the chromatographic plate. It is not easy to dissolve such an extract completely, even with use of a vortex mixer, in less than 100 μL and, if this, no more than 5 μL can be reproducibly spotted at each application. Repeated spotting is time-consuming and very subject to operator error.

We have found that only 5 to 10 μL of solvent need be added to the extract, if this is sited in a round-bottomed tube. The solvent is then brought into contact with all the extract by using a relatively thick, round-bottomed glass rod, which traps the solvent between itself and the inside wall of the tube by surface tension. The solution of extract in solvent, which then becomes spread over the wall of the tube, can then be collected by brief centrifugation into the bottom of the tube, from whence it can be drawn into the capillary used for spotting.

The ideal solvent or solvent mixture should have a low volatility, should dissolve most or all of the dried extract, and should be chosen so that the compound to be assayed has a low Rf value were the solvent to be used to develop it on a thin-layer chromatographic plate. For the strongly-basic β-blockers we have found that an equimol mixture of butanol-1-ol and acetic acid mixture fits these conditions. Although the mixture is not too volatile, it evaporates readily from a thin-layer chromatographic plate, especially if the plate is conditioned by exposure to reduced pressure before elution.

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Possible Interference with the Helena Laboratories' Method for Phosphatidylglycerol

To the Editor:

Evaluating Helena Laboratories' "Fetal-Tek 200" method for the lecithin/sphingomyelin (L/S) ratio and phosphatidylglycerol (PG), we observed major discrepancies with our own thin-layer chromatographic method for PG. Our method (Table 1), a modification of a procedure developed by S. Faro at Louisiana State University Medical Center in New Orleans (1), has been successfully used for more than a year in our laboratory. The results we obtained with the two methods for 38 amniotic fluids included 26 samples that were positive for PG by both methods; however, 10 of 12 samples that were negative for PG by our method were positive by the Helena method. Only two samples were negative by both methods. Expressing the PG as the PG/S ratio or as percent PG to total phospholipids did not improve the poor correlation.

To determine what was causing the problem we did several experiments. First, to determine if the Helena method had better sensitivity, we chromatographed various amounts of PG standard by both methods. To the contrary, we found our method was more sensitive (78 ng vs 312 ng). To determine if some substance was co-chromatographing with PG on the Helena plate we conducted the following experiment: One of the spurious samples was extracted and, according to the Helena procedure, chromatographed along with a PG standard in a separate channel. The plate was then dried and cut in half length-wise. The half corresponding to the standard was charred to locate the PG spot; the other half was left uncharred. The silica on the plate containing the sample was then scraped off in the area corresponding to the Rf of the PG standard. This silica was then eluted with chloroform, filtered, and spotted on our silica plate. We then carried out our procedure as usual. After charring, we observed that the sample contained a spot with a different Rf (0.25) from that of the PG standard (0.18).

We have only limited clinical information at this time to determine the clinical significance of this finding. Of the 10 patients for whom results were spurious, only two delivered within a day of the amniocentesis. One of the newborns developed mild respiratory distress, the other apnea and bradycardia, possibly secondary to a patent ductus arteriosus. Two more patients delivered healthy babies at later dates, but these patients had positive PGs by the time of delivery. The other six women were delivered at later dates with no PG followup; all delivered healthy babies.

In a study of 62 patients, Huang et al. found good clinical agreement with the PG/S ratio, using Helena's procedure (2). Despite this, at this time we recommend caution in interpreting PG by Helena's Fetal-Tek 200 procedure. Work is now underway to identify the interfering substance and determine its clinical significance.

References


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A spokesman for Helena Laboratories responds:

To the Editor:

We appreciate and share your correspondents' concern for accurate results in this crucial test.

Our package insert explains that the primary purpose of our procedure is quantitation of the L/S ratio with simultaneous separation of the four other known phospholipids. A marker is used to indicate the relative position of the phospholipids on our TLC plates developed with our mobile phase.

Qualitative results for PG that may be obtained with Fetal-Tek 200 should be used with caution because of the difference in individual laboratories' interpretation of the results and the lack of conclusive studies showing that PG is the only component migrating in this area.

Since the materials have been made available, two papers were presented at the AACC national meeting indicating excellent correlation between the Fetal-Tek 200 L/S procedure and other L/S methods (Clin Chem 28:1637 and 1661, 1982). The latter of these papers indicated that they had "difficulty" in interpreting the data for PG by the

Table 1. Summary of PG Method Used at Earl K. Long Memorial Hospital

| 1. Extraction identical to Helena Labs'. |
| 2. Extract spotted on silica gel GHL plates (250-μm, 5 x 10 cm) (Analtech, Inc., Newark, DE 19711). |
| 3. Migrated in a cylindrical chamber (Analtech) in the following solvent system: 8 ml tetrahydrofuran, 6.4 ml methyl (dimethoxyxymethane), 1.6 ml methanol, and 0.9 ml of 7.9 mol/L ammonium hydroxide. |
| 4. Plate is charred 10 min at 325 °C after dipping in a 30 g/L solution of cupric acetate in 80 g/L phosphoric acid. |

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