Sample and SPE Extraction

(**a**) *Sample extraction method.*—Grind ca 5 g comfrey root and pass the material through a U.S. Standard #40 sieve prior to weighing. Weigh ca 1.0 g comfrey root powder into a 200 mL centrifuge bottle, and add 100 mL extraction solvent (extraction solvent components become miscible following addition to plant material, due to residual water). Place the bottle in an ultrasonic bath for 15 min and agitate for 30 min with a wrist-action shaker. Centrifuge for 5 min at ca 2200 rpm to clarify the extracts. Filter the extract through Whatman 1PS filter paper to remove residual water.

(**b**) *SPE method.*—Quantitatively transfer 90 mL sample extract to the Ergosil column under vacuum. Wash the column with 2 mL acetone–chloroform (8 + 2, v/v) under vacuum to remove pigments. Remove residual chloroform with 2 mL petroleum ether. Continue applying vacuum until the column is no longer cool to the touch, indicating that petroleum ether is removed. Elute PAs under vacuum with 2 successive 1 mL aliquots methanol into 2 mL volumetric flask until the column is dry. Dilute to volume with methanol, filter the sample (0.45 mm PTFE) into an autosampler vial, and analyze by LC using the previously described parameters. Store final extracts under refrigerated conditions.

(**c**) *SPE recovery testing of alkaloids.*—In triplicate, weigh 1.0, 5.0, 10.0, and 25.0 g into individual 200 mL centrifuge bottles. Extract samples with 100 mL extraction solvent as previously described. Filter the extracts through Whatman 1PS filter paper to remove residual water. From the individual samples, quantitatively transfer 25 mL sample extract to a suitable container and clean up using the Ergosil SPE procedure as previously described. Quantitatively remove an additional 25 mL sample extract and evaporate to dryness. Redissolve the sample in exactly 2 mL methanol. Filter all samples (0.45 mm PTFE) into autosampler vials and analyze by LC using previously described parameters.

(**d**) *Extraction recovery optimization.*—In triplicate, weigh 1.0, 5.0, 10.0, and 25.0 g into individual 200 mL centrifuge bottles. Extract samples with 100 mL extraction solvent as previously described. Filter the extracts through Whatman 1PS filter paper to remove residual water. Quantitatively remove 25 mL from each extract, evaporate to dryness, and redissolve in exactly 2.0 mL methanol. Filter the samples (0.45 mm PTFE) into autosampler vials, and analyze by LC using the previously described parameters.

(**e**) *Extraction efficiency testing.*—Weigh 10 replicates of 1.0 g material into individual 200 mL centrifuge bottles. Extract samples with 100 mL extraction solvent as previously described. Remove 5 mL extract, evaporate to dryness, and redissolve in exactly 2.0 mL methanol. Remove an additional 45 mL from each extract and discard. Add 50 mL fresh extraction solvent to each replicate, and reextract as previously described. Remove an additional 5 mL extract, evaporate to dryness, and redissolve in exactly 2.0 mL methanol. Filter samples (0.45 mm PTFE) into autosampler vials, and analyze by LC using the previously described parameters.

(**f**) *Ruggedness.*—The Ergosil SPE method was evaluated by 3 analysts over a 2-year period, with comfrey root extractions ranging from 1 to 40 g in 100 mL (0.125 to 15.0 g equivalents) through Ergosil handpacked columns. Overall percentage recovery in all cases was >90.