

3.3.2.2 Clean-Up of Extract

The first clean-up step was performed on a silica gel column. Approximately 400 mg of silica gel (Kieselgel 60, 70-230 mesh ASTM; Merck, Darmstadt, FRG) was loaded into a Pasteur pipette plugged with glass wool, which had been pre-washed with methanol. 3 ml of toluene were pipetted on to the silica gel and allowed to drain until the solvent level reached the surface of the gel.

The residue from the sample extraction step (section 3.3.2.1) was taken up in approximately 0.2 ml CH_2Cl_2 and loaded on to the silica gel column. The sample tube was rinsed with 0.2 ml CH_2Cl_2 and this was also added to the column. When the solvent level had reached that of the gel, 0.5 ml of toluene-acetone (95:5, v/v) was pipetted on to the gel, followed by a further 3 ml. The collection tube was changed and 4 ml of dichloromethane-methanol (95:5, v/v) was added. The column was allowed to drain completely and this second fraction was taken to dryness under a stream of nitrogen at 40°C.

The second clean-up step used a charcoal-alumina column. Equal weights of charcoal (non-activated, Darco G-60, J.T. Baker Chem. Co., Phillipsburg, New Jersey, USA) and