Lithocholic acid: notes on purification

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A survey of several commercial samples of lithocholic acid (**Table 1**) by gas–liquid chromatography indicated that they all had an impurity, with an RRT = 0.36, varying in amounts from 3 to 6% of the lithocholic acid peak.

Using the lithocholic acid obtained from Sigma Chemical Co. (No. L-6250), we have devised a simple recrystallization method that provides lithocholic acid that is 99.8% pure by gas–liquid chromatography.

The principle is that one should use combinations of solvents in which the impurities are successively more soluble and less soluble than the compound. Lithocholic acid and its methyl ester derivative crystallize so easily from methanol–water that one can be deceived into thinking that the impurities are no longer present. Actually, because the impurity (RRT = 0.36) is less soluble than lithocholic acid, it tends to co-crystallize with it. Instead of using combinations of solvents, it is also possible to use a single solvent and depend on the difference in solubility of the compound and the impurity at different temperatures.

After converting the lithocholic acid to the methyl ester and crystallizing from methanol–water, it was recrystallized once from heptane–ethyl acetate. It was then acetylated and the acetate crystallized twice from boiling absolute ethanol. Following hydrolysis to the free acid with 5% methanolic KOH, it was crystallized initially from methanol–water and recrystallized from boiling ethyl acetate. The final product crystallized very readily as small clusters of needles and contained 0.24% of impurity (RRT = 0.36). Additional crystallizations from ethyl acetate can further reduce the impurity. On three occasions, 1 g of lithocholic acid yielded 61%, 64%, and 62% as a crystalline compound containing 0.24% of impurity or less.

Direct probe mass spectrographic analysis of the lithocholic acid from Sigma Chemical Co. before and after crystallization using negative chemical ioniza-

TABLE 1. Percent impurity in lithocholic acid

Source	Impurity[a]	Melting Point[b]
	%	°C
Fluka	5.7	184–186
Aldrich	6.2	184–186
Calbiochem	7.2	184–186
Supelco	3.4	186–188
N.C.I.[c]	4.6	184–186
Sigma	5.1	184–186
Sigma recrystallized[d]	0.2	188–189

[a] RRT = 0.36.

[b] Melting point (uncorrected): A Thomas-Hoover apparatus was used and samples were done simultaneously. According to the literature (Ref. #2) melting points are 185–186°C (aqueous alcohol), 188°C (aqueous acetone or ether–petroleum ether), 188°C (aqueous methanol).

[c] Sample given by National Cancer Institute to Dr. Ernest Bueding, Johns Hopkins University School of Hygiene and Public Health.

[d] As described in manuscript.

Abbreviation: RRT, relative retention time.

tion (1) indicates that the impurity has a molecular weight of 132.

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