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Note

Purification of quinolines for bioassay by preparative liquid chromatography

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While mutagenicity tests utilizing bacterial strains and cell culture transformations are widely used as pre-screens for carcinogens, bioassays in laboratory animals remain the definitive method of determining the carcinogenicity of chemicals. Since impurities may modify the toxicity and carcinogenicity of a test compound, it is important to set high standards of purifications for bioassays for carcinogenicity[1]. Purity criteria of greater than 99.9% are required for many critical structure–activity studies[2]. Unless the environmental chemical to be tested is a technical product, chemicals of less than 99% purity should not be used in bioassays. Fractional distillation and crystallization are sometimes adequate for the purification of synthetic chemicals. However, refined techniques, such as zone refining, spinning band distillation, preparative gas chromatography and various forms of liquid chromatography (LC) are more often required to yield chemicals of sufficient purity. In this communication we will describe a method for the purification of quinolines for bioassay, using preparative liquid chromatography (Prep LC). This technique is preferable to conventional LC in terms of capacity and resolution, and it is most valuable in isolating gram quantities of highly purified materials from natural sources, such as plant extracts, coal tar, etc.

Quinoline was recently found to be a rat liver carcinogen[3] and a mutagen in *Salmonella typhimurium*[4,5]. It is formed by pyrosynthesis during the combustion of nitrogen-containing organic compounds and is, therefore, present in the suspended particulate matter of New York City air (20–70 ng per 1000 m^3)[6] as well as in the mainstream (0.6–2.0 μg per cigarette) and sidestream (18–200 μg per cigarette) smoke of tobacco products[7]. Since quinoline is also an important industrial chemical its reported tumorigenicity requires further delineation. Currently, we are testing quinoline and methylquinolines for carcinogenicity and cocarcinogenicity on mouse skin at 3 dosage levels (0.2, 1 and 3%)[8]. This required about 40 g of quinoline and 10 g of each of several alkylquinolines in purities greater than 99%. All of these quinolines were commercially available, and analysis revealed that some alkylquinolines were contaminated with isomeric products. The use of Prep LC followed by fractional vacuum distillation allowed rapid and efficient purification of these chemicals in quantities sufficient for bioassay.

EXPERIMENTAL

Materials

Quinoline (reagent grade) was obtained from Fisher Scientific (Fair Lawn, N.J., U.S.A.). 2-Methylquinoline (quinaldine), 4-methylquinoline (lepidine), 8-methylquinoline and 2,4-dimethylquinoline were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Purities of these commercial-grade quinolines are listed in Table I. These vary from 91 to 99.5% as determined by gas chromatography (GC). All solvents were of spectrograde quality from Fisher Scientific or Burdick & Jackson (Muskegon, Mich., U.S.A.).

TABLE I

PURIFICATION OF QUINOLINES FOR BIOASSAY

Chemical	*Purity (%) as determined by GC–FID*			*Prep LC solvent program*
	Before Prep LC	*After Prep LC*	*After Prep LC and vacuum distillation*	
Quinoline	99.5	99.9	100	Acetonitrile–chloroform (1:9)
2-Methylquinoline	93.8	98.03	99.4	Acetonitrile–chloroform (1:9)
4-Methylquinoline	99.0		99.6*	Not used
8-Methylquinoline	98.02	99.94		Chloroform + 1% ethanol
2,4-Dimethylquinoline	91.5	97.3	99.0	Acetonitrile–chloroform (1:9)

* Not purified by Prep LC.

Apparatus

Thin-layer 250-μm silica precoated glass plates from Brinkmann (Westbury, N.Y., U.S.A.) and μ-Porasil high-performance LC (HPLC) adsorption columns (Waters Assoc., Milford, Mass., U.S.A.) were used to explore solvent systems for Prep LC.

GC was used for quantitative assays of quinolines both before and after purification. A 6 ft. × 1/8 in. O.D. stainless-steel column packed with 10% Carbowax 20M–terephthalic acid on Gas-Chrom Q (60–80 mesh) was used in a Hewlett-Packard 5710A GC instrument with flame ionization detector (FID). The detector output was fed into a Hewlett-Packard 3380S integrator for automatic print-out of peak areas.

For the actual purification of the quinolines, a Prep LC System 500 (Waters Assoc.) unit with solvent delivery system (up to 0.5 l/min) and differential refractometer was employed. Self-contained cartridges made from high-density polyethylene 5.7 × 30 cm, fitted with stainless-steel frits at both ends and filled with 316 g of 80 μm nominal diameter silica particles were used as columns. These cartridges (PrepPak-500/Silica from Waters Assoc.) require radial compression by 500 p.s.i. of nitrogen pressure in a chamber for conversion into efficient columns. This Prep LC system has been described in detail elsewhere[9].

After Prep LC, most of the quinolines were further purified by fractional vacuum distillation (0.02–0.1 mm Hg).

Procedure

Each batch of quinoline obtained from commercial sources was assayed quantitatively by GC. The solvent system for Prep LC was determined for each quinoline by thin-layer chromatography (R_F values 0.25–0.4) and by analytical HPLC. Solvent mixtures were chosen in an effort to economize on solvents while maintaining adequate resolution. About 10-g samples were injected for each separation and effluent fractions were collected, concentrated and assayed by GC.

RESULTS AND DISCUSSION

Our goal was the preparation of 10–40 g of five different quinolines of adequate purity for bioassay. Table I summarizes the results of the Prep LC purification. In all cases adequate purities were obtained after Prep LC and vacuum distillation. 2-Methylquinoline and 2,4-dimethylquinoline required more extensive work since the commercial samples contained several isomers and various other impurities. 2,4-Dimethylquinoline was not purified further due to lack of starting materials. Fig. 1 shows a GC tracing of 8-methylquinoline before and after purification by Prep LC while Fig. 2 shows a Prep LC tracing of a 10-g injection of 8-methylquinoline. The clean-up of 10 g of 8-methylquinoline was achieved in less than 10 min, a good indicator of the capabilities of Prep LC. This technique allowed us to purify 4 quino-

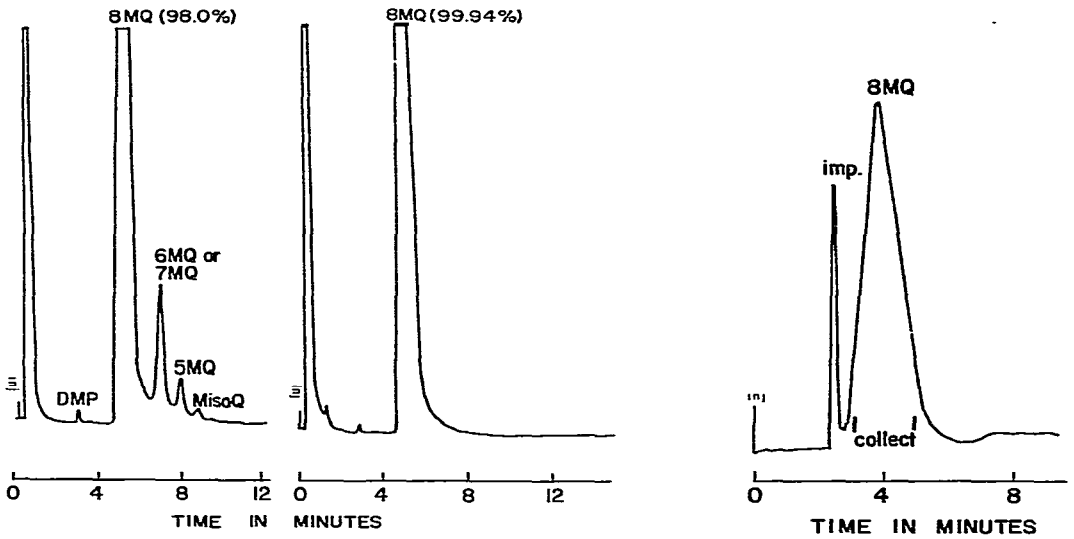

Fig. 1. Gas chromatographic traces of 8-methylquinoline (8MQ) before (left) and after (right) Prep LC purification. MisoQ = methylisoquinoline; DMP = dimethylpyridine.

Fig. 2. Preparative LC of a 10-g sample of 8-methylquinoline (8MQ). Chromatographic conditions: column, one cartridge of PrepPak-500/Silica; solvent, 1% ethanol in chloroform; flow-rate, 0.3 l/min; detection, refractive index.

lines within 3 h. On conventional LC these separations would require several days. To economize on cost and solvents, one cartridge was used for all quinolines and cross contamination was not encountered. The use of such a Prep LC system is expected to save time, effort and solvents for similar separations and clean-up problems.

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