PURIFICATION OF TRITIATED PHENACETIN AND N-ACETYL-p-AMINOPHENOL .

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summary

Phenacetin and N-acetyl-p-aminophenol (NAPA) tritiated by catalytic exchange (1), were quite impure as purchased. No previous reports on purification of these radioactive compounds could be found, although there are reports of separation of phenacetin or NAPA by chromatography (2, 3). Purification was carried out in two steps : gel filtration with Sephadex G-10, followed by thin layer chromatography on silica gel.*

MATERIALS AND METHODS.

A 0.9 x 60 cm column† was filled with 40-120 μ Sephadex G-10 gel†, with glass distilled water as the eluant. When packing was complete, the height of the Sephadex bed was 56 cm. A flow rate of 5.2 ml/hour was maintained with a peristaltic pump‡. The column effluent, which was monitored at 254 nm§, was collected in 1.3 ml fractions||.

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* New England Nuclear Corp., Boston, Mass. 02118.

† Pharmacia (Canada) Ltd., Montreal, Quebec.

‡ LKB Perpex Peristaltic Pump, LKB Produkter, Bromma, Sweden.

§ Uvicord II, Ultraviolet Absorptometer Type 8303A, LKB Produkter, Bromma, Sweden.

|| UltroRac Fraction Collector, LKB Produkter, Bromma, Sweden.

** Eastman Kodak Company, Rochester, N.Y.: Silica Gel 6061; developing apparatus 6017

Thin layer chromatography was carried out on Eastman** silica gel chromatogram sheets, which were developed with the solvent system chloroform: acetone: cyclohexane (50:30:20) in an Eastman chromatogram developing apparatus. The solvent system benzene: ether: methanol: acetic acid (60:30:5:5) was used when a second dimension was developed.

The specific activities of the tritiated phenacetin and NAPA were 10 and 22 mCi/mg respectively. Samples of each tritiated drug as supplied in methanol, were dried under nitrogen, redissolved in 0.5 ml distilled water and then applied to the Sephadex column. The effluent was collected for 17.5 hours in 70 fractions, or until a total of 91 ml of effluent had been collected. A 10 µl sample from each fraction was added to 10 ml of Bray's solution[4] and counted in a Philips* Liquid Scintillation Analyzer. The fractions containing radioactivity were identified and those related to either the ^{3}H-phenacetin or ^{3}H-NAPA were pooled. The solvent was evaporated by heating at 70°C under nitrogen, and the residue was redissolved in 0.5 ml of methanol and further purified by thin layer chromatography. Aqueous solutions of chemically pure unlabelled phenacetin and NAPA (50 µg/ml) were also subjected to gel filtration under similar conditions to provide reference standards for location of the corresponding peaks of the labelled compounds. The position of the peaks was determined by UV monitoring and spectrophotometry at 254 nm.

Sample aliquots of 0.1 ml were streaked on to chromatography plates by means of a micro-pipette. The chromatograms were developed in the solvent system chloroform: acetone: cyclohexane (50:30:20) using ascending chromatography. The positions of the narrow bands associated with radioactive and non-radioactive phenacetin or NAPA were visualized under UV light, compared, and their outlines marked. The separated compounds were eluted off the silica material with

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* Philips Co., Eindhoven, The Netherlands, Model PW 4510.

5 ml methanol by a spot collector attached to a vacuum head*. The suspension was filtered through a cellulose membrane of 0.25 μ pore size*. The filtered solvent was evaporated by heating at 70°C under nitrogen and the residue redissolved.

The purity of the resulting tritiated phenacetin or NAPA was checked by comparison with chemically pure unlabelled reference standards, using two dimensional thin layer chromatography and autoradiography. In the latter procedure, X-ray film in a cassette was exposed to each chromatogram for 4 days.

RESULTS AND DISCUSSION.

An autoradiograph of unpurified tritiated NAPA following thin

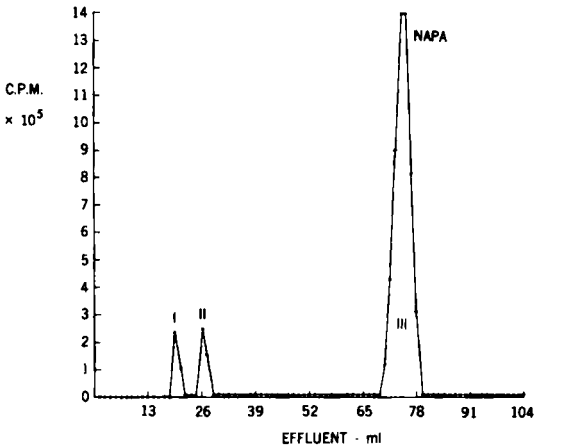

Figure 1: Elution curve of ^{3}H-NAPA from a Sephadex G-10 gel bed. Fraction III represents the drug and fractions I and II are due to impurities.

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* Brinkman Instruments (Canada) Ltd., Rexdale, Toronto.

layer chromatography in two dimensions showed 10 spots. A similar preparation of phenacetin showed 4 spots. The compounds had been tritiated about two years prior to this analysis. After separate gel filtration of these compounds on Sephadex G-10, NAPA and phenacetin were eluted as well defined peaks (Figs. 1 and 2, peak III), distinct from other peaks representing impurities (Figs. 1 and 2, peaks I and II). The labelled impurities associated with tritiated NAPA and phenacetin had radioactivity peaks that were similar (peaks I & II, Figs. 1 and 2), but were not associated with UV absorption peaks.

When aliquots of the fractions collected after gel filtration corresponding to either the phenacetin or NAPA peaks were pooled, analysis by two-dimensional chromatography and autoradiography showed

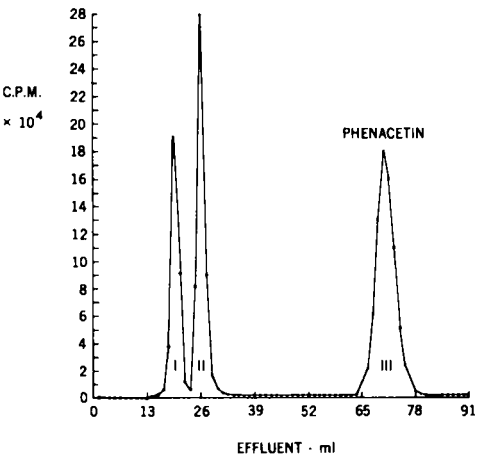

Figure 2: Elution curve of ^{3}H-phenacetin from a Sephadex G-10 gel bed. Fraction III represents the drug and fractions I and II are due to impurities.

that phenacetin and NAPA were purified to 96.7 and 98% respectively, as shown by comparison of the radioactivity in the major and minor spots which occurred in each case. However, a second run in two dimensions of the material eluted from the major spot on silica gel, in each case after chromatography in one dimension, yielded only one spot which had the same Rf values as the chemically pure unlabelled compounds. The solvent system chloroform: acetone: cyclohexane gave separation characterized by Rf values of 0.78 and 0.47 for phenacetin and NAPA respectively; and the solvent system benzene: ether: methanol: acetic acid gave values of 0.68 and 0.43.

Recoveries were 29 and 26% of total radioactivity for pure tritiated phenacetin and NAPA, respectively, after gel filtration and chromatography on silica gel in one dimension.

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