3. The TLC plates may absorb plasticizers from shipping boxes after lengthy storage, causing yellowish discoloration along the sides of the plate's silica gel surface. Either avoid lengthy storage or store in glass containers.
4. Acidification of eluate prior to evaporation is not necessary if proper control of temperature is maintained.
5. All barbiturates and glutethimide migrate to approximately the same R_f.
6. Constant attention to and knowledge of excreted metabolites increases the confidence limits in tentative identifications.

Summary

A thin-layer chromatographic system, characterized by a development system designed to offset humidity factors and to effect distinct separations of a significant number of drugs subject to abuse, is presented.

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A Death Due to Levorphanol

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Abstract

A death due to levorphanol, 3-hydroxy-N-methylmorphinan, is described. Levorphanol concentrations were measured in antemortem blood and postmortem tissue using thin-layer and gas chromatography equipped with a nitrogen-phosphorous detector.

Case History

A 61-year-old white male was diagnosed as having chronic lymphocytic leukemia. Between 8:00 and 9:00 A.M. on the day in question, he took 15 2 mg tablets of L-Dromoran® . Approximately 45 minutes later, the patient appeared "drunk." The family physician was notified, and the patient was admitted to a hospital at approximately 4:00 P.M.

During the evening, the patient became hypotensive, lethargic, and diaphoretic. During the early morning hours the patient's respirations became shallow and shortly thereafter, ceased. The patient was pronounced dead at 2:52 A.M.

The body was embalmed before the autopsy was conducted; however, an antemortem blood specimen, liver, bile, and kidney were submitted for analysis.

Pathology

The body was that of a 61-year-old white male weighing 130 pounds and measuring 68 inches.

Pleural cavities contained less than 25 cc of fluid with moderate fibrous adhesions in the right pleural cavity. The heart weighed 300 grams. The valves were unremarkable, and the coronary arteries were devoid of atherosclerotic changes. The right lung weighed 350 grams, and the left lung weighed 300 grams. The pleural surfaces of both lungs bulged from numerous emphysematous blebs.

The liver weighed 2100 grams and had a light tan smooth capsular surface. The gallbladder contained 50 cc of dark green bile and was unremarkable.

There was abundant evidence of chronic lymphocytic leukemia with leukemic cell infiltration into the lymph nodes, liver, spleen, kidney, prostate, epicardium, and lungs.

Toxicology

Chemicals, Reagents, and Solutions

Inorganic chemicals were analytical, reagent grade. Solutions were prepared in distilled water and stored in glass containers.

Organic chemicals were distilled in glass and obtained from Burdick & Jackson Laboratories, Inc.

Levorphanol was a gift from Roche Laboratories (Nutley, NJ). A stock standard was prepared in methanol to contain 1.0 mg/mL levorphanol (free base). Working standards used in preparing the standard curve were made by diluting the stock solution to concentrations of 5, 10, 15, and 20 μg/mL, respectively.

The internal standard was made to contain 100 μg/100 μL codeine (free base) in methanol.

Thin-Layer Chromatography

TLC plates used were Quantum LQ6F, 250 micron, 20 x 20 cm available through Bodman Chemical Co., Atlanta, GA.

The mobile phase was prepared according to Cochin and Daly (1) and consisted of benzene:dioxane:ethanol:concentrated ammonium hydroxide (50:40:5:5).

The TLC tank was lined and sealed with a water soluble paste and allowed to equilibrate overnight (2).

Gas Liquid Chromatography (GLC)

GLC data was obtained on a Hewlett-Packard Model No. 5710 gas chromatograph with a dual nitrogen-phosphorous specific detector. The operating parameters were as follows:

Column A

1.2 m x 4 mm o.d. silanized glass column, packed with 3% OV-101 on 100/120 mesh Chromosorb W HP
Carrier gas: Helium
Carrier flow: 25 mL/minute
Column temperature: 210°C
Injection port temperature: 250°C
Detector temperature 300°C
Voltage to collector electrode: Optimum.

Column B

1.2 m x 4 mm o.d. silanized glass column, packed with 3% SP 2250 DB on 100/120 mesh Supelcoport
Carrier gas: Helium
Carrier flow: 25 mL/minute
Column temperature: 210°C
Injection port temperature: 250°C
Detector temperature: 300°C
Voltage to collector electrode: Optimum.

Extraction, Isolation, and Identification

A 5-mL aliquot of blood and bile were diluted 1:1 with distilled water.

A 10-gram sample of liver and kidney was homogenized with an equal amount of distilled water. The specimens were acidified with 1.0 mL of concentrated H_2SO_4 and hydrolyzed in a boiling water bath for 30 minutes. The hydrolyzed specimens were cooled to room temperature and centrifuged at 2000 rpm for five minutes. The supernatant was filtered through Whatman No. 1 filter paper. The tissue remaining after centrifugation was washed with 5 mL 1.0 mol/liter H_2SO_4, centrifuged for five minutes at 2000 rpm, and filtered. The combined filtrates were transferred to a 125-mL separatory funnel and extracted two times with 50 mL ethyl ether. The acid ether extract was analyzed by thin-layer chromatography with negative results.

The aqueous acidic phase was made alkaline with concentrated NH_4OH, and extracted two times with 50 mL of a 9:1 chloroform:isopropanol mixture. The organic phases were combined and extracted with 10 mL 3% NaOH and twice with 10 mL distilled water (3). The organic phase was dried with anhydrous Na_2SO_4, filtered, and evaporated to approximately 10 mL at 60°C, and allowed to evaporate to dryness at room temperature.

The tissue extracts were streaked on the absorbent layer of the TLC plate adjacent to a mixture of standards containing morphine, codeine, meperidine, oxycodone, levorphanol, pentazocine, and hydromorphone. The plate was allowed to develop over 15 cm, then removed from the tank and air dried.

The portion of the plate containing the sample was covered with a glass plate, and the remainder of the plate was sprayed with acidified iodoplatinate (4).

The R_f and relative R_f (codeine) values for the standards are shown in Table I.

The area adjacent to the levorphanol standard and with a R_f range of ±5 was scraped from the plate and transferred to a 60-mL separatory funnel containing 5 mL of 50% NH_4OH and 200 nanograms codeine.

The aqueous alkaline solutions were extracted two times with 25 mL chloroform:isopropanol (9:1). The combined extracts were dried with anydrous sodium sulfate, filtered, and evaporated to near dryness on a steam bath to approximately 5 mL. The remaining solvent was evaporated to dryness at room temperature under a stream of nitrogen. The residue was dissolved in 50 microliters of methanol.

A 1.0-microliter aliquot of the reconstituted residue was injected into the gas chromatograph on columns A & B. Retention times and relative retention times to codeine are shown in Tables II and III.

A standard curve was prepared by plotting R, which is the ratio of peak heights of levorphanol and codeine versus concentration of levorphanol in μg/mL. Figure 1 represents an average curve for three sets of determinations.

The concentrations of levorphanol in the tissues were determined from the standard curve and are shown in Table IV.

Table I. Thin-Layer Chromatographic Data

Compound	*R_f x 100*	*rR_f*
Morphine	10	0.34
Hydromorphone	11	0.38
Levorphanol	19	0.60
Codeine	29	1.00
Oxycodone	58	2.00
Meperidine	64	2.21
Pentazocine	71	2.45

Table II. Gas Chromatographic Retention Data of a Number of Analgesics on Column A (3% OV-101)

Compound	*R_t*	*rR_t*
Morphine	8.89	1.47
Hydromorphone	7.42	1.23
Levorphanol	3.78	0.63
Codeine	6.03	1.00
Oxycodone	9.55	1.58
Meperidine	0.95	0.16
Pentazocine	4.63	0.77

Table III. Gas Chromatographic Retention Data of a Number of Analgesics on Column B (3% OV-17)

Compound	*R_t*	*rR_t*
Morphine	9.30	1.25
Hydromorphone	8.64	1.16
Levorphanol	3.98	0.54
Codeine	7.42	1.00
Oxycodone	10.20	1.37
Meperidine	0.60	0.08
Pentazocine	4.10	0.55

Discussion

Levorphanol, a narcotic analgesic, is a congener of morphine. The compound possesses an analgesic potency approximately four times that of morphine. The increase in analgesic

Table IV. Tissue Concentrations of Levorphanol in a Fatal Case

Tissue	*Concentration*
Blood (antemortem)	0.8 mg/liter
Bile*	15 mg/liter
Liver*	11 mg/kg
Kidney*	1 mg/kg

*Embalmed

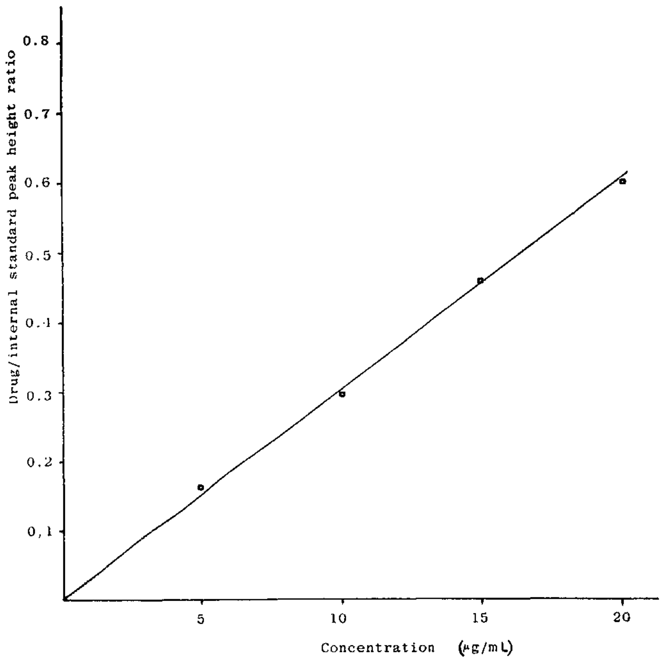

Figure 1. Drug/internal standard ratio vs. drug concentration of 5.0-20.0 µg/mL.

potency is accompanied by a proportionate increase in the drug's ability to decrease respiration (5). Little data is available on toxic concentrations of levorphanol in man (6,7).

Given a history of the number of levorphanol tablets consumed, the clinical profile prior to death, the absence of any other drugs, and the levorphanol concentrations in the tissues examined, it was determined that the cause of death was the result of ingestion of a lethal dose of levorphanol; and the manner of death was accidental.

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