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Note

Determination of levorphanol (Levo-Dromoran®) in human plasma by combined gas chromatography—negative ion chemical ionization mass spectrometry

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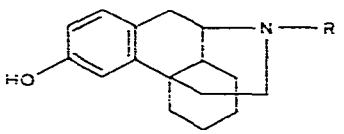
Levorphanol (17-methyl-morphinan-3-ol, Levo-Dromoran®) is a synthetic narcotic analgesic which is more potent than morphine when administered orally, parenterally or subcutaneously [1].



R = CH_3 levorphanol
R = C^2H_3 levorphanol—d_3

Both levorphanol and its metabolite, norlevorphanol, are excreted principally as glucuronide conjugates [2—4]. A recently reported radioimmunoassay (RIA) [5, 6] is the only available assay for measuring plasma concentrations of levorphanol, in spite of the fact that the drug has been marketed for over 25 years. This paper reports a gas chromatography—mass spectrometry (GC—MS) assay for levorphanol which was developed to quantitate plasma concentrations of levorphanol generated following the administration of Levo-Dromoran to man. The assay features the use of a trideuterated analogue of levorphanol (levorphanol-d_3) as the internal standard, conversion of levorphanol to a derivative with electron-capturing properties and ionization of the derivative by methane negative chemical ionization.

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EXPERIMENTAL

Gas chromatograph

A Finnigan Model 9500 gas chromatograph is equipped with a glass column (120 cm × 2 cm I.D.) packed with 3% poly S-176 on 80—100 mesh high-performance Chromosorb W (Applied Science Labs., State College, PA, U.S.A.). The column was conditioned overnight at 300°C with a nitrogen flow-rate of 40 ml min^{-1}. Methane (101 kPa) was used in the assay as both GC carrier gas and negative ion reagent gas. The temperatures of the injection block, GC oven and GC—MS transfer line were 290°C, 280°C, and 280°C, respectively. Under these conditions the pentafluorobenzoyl derivative of levorphanol had a retention time of 2 min.

Mass spectrometer

A Finnigan Model 3200 quadrupole mass spectrometer was set to give the maximum response consistent with reasonable ion peak shape and unit resolution. The modification to the instrument to permit the detection of negative ions have been described [7]. The methane ion source pressure was 66.7 Pa. The ion source was operated at an emission of 0.25 mA, an ionizing voltage of 120 V and without any external heating. The voltages on the conversion dynode and electron multiplier were +2.5 and —2.0 kV, respectively. The preamplifier was set to give 10^{-8} A V^{-1}.

Data system

Selected ion monitoring measurements were made using a Finnigan Model 6000 data system with revision I software. A scan of *m/z* 451 and *m/z* 454 was made in 1 sec.

Glassware

Sixteen-ml culture tubes (Pyrex 9825) provided with Teflon®-lined screw caps were used for plasma extractions. Five-ml conical centrifuge tubes (Pyrex 8061) were used for the derivatization procedures and for the final evaporation of the derivatived extract. All tubes, after washing with detergent and water, were treated with Siliclad® (Clay Adam, Parsippany, NJ, U.S.A.) and were finally rinsed with methanol and dichloromethane (Fisher Scientific, Pittsburgh, PA, U.S.A.) just prior to use.

Solvent evaporator

Solvents were removed at 60°C under nitrogen (N-Evap, Organomation Assoc., Worchester, MA, U.S.A.).

Shaker

Extractions were performed by shaking (60 strokes min^{-1}) on a variable-speed reciprocating shaker (Eberbach, Ann Arbor, MI, U.S.A.).

Centrifuge

A Damon/IEC (Needham, MA, U.S.A.), Model CRU-500 refrigerated centrifuge was operated at 1320 *g* and 10°C.

Scintillation counting

A Packard Tri-Carb Model 3380 scintillation counter was used with an external standard.

Chemicals

Levorphanol tartrate was supplied by Dr. W. Scott, Chemical Research Department, Hoffmann-La Roche, Nutley, NJ, U.S.A. Levorphanol-d_3 hydrochloride was synthesized using the method of Ellison et al. [8]. Tritiated levorphanol was provided by Drs R. Muccino and J. Cupano, Hoffmann-La Roche. Methanol, dichloromethane, chloroform and benzene were supplied by Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Pentafluorobenzoyl chloride and 4-dimethylaminopyridine were purchased from PCR Research Chemicals (Gainesville, FL, U.S.A.) and Aldrich (Milwaukee, WI, U.S.A.), respectively.

Solutions

Borate buffer (pH 9) was prepared as previously described [9]. Stock solutions (mg/ml) of levorphanol tartrate and levorphanol-d_3 hydrochloride were prepared in methanol. Aliquots of the stock solution were diluted with methanol to give working solutions containing 100 ng ml^{-1} of levorphanol-d_3 free base and either 0 (solution A), 10 (solution B), 50 (solution C), 100 (solution D), 200 (solution E), or 500 ng ml^{-1} (solution F) of levorphanol free base.

Procedure

Levorphanol-d_3 (10 ng) was added to 1 ml of either the calibration curve plasma samples, i.e., drug-free control plasma, or experimental plasma samples, and the mixture was vortexed briefly. Duplicate calibration curve plasma samples were spiked with 0.1 ml of either solution A (0 ng of levorphanol), B (1 ng of levorphanol), C (5 ng of levorphanol), D (10 ng of levorphanol), E (20 ng of levorphanol), or F (50 ng of levorphanol). One ml of 1 *M* borate buffer (pH 9) was added and the samples were extracted with 7 ml of benzene—dichloromethane (9:1) by shaking for 50 min. The samples were then centrifuged for 10 min and the organic layer was transferred, a portion at a time, to a 5-ml centrifuge tube, followed by evaporation of the solvent. The organic solvents were then evaporated to dryness. The residue was dissolved in 100 μl of chloroform and derivatization was accomplished by adding 20 μl of 1% 4-dimethylaminopyridine in chloroform and 20 μl of pentafluorobenzoyl chloride. After 2 h at room temperature, this solution was washed with 1 ml of borate buffer (pH 9) and was extracted with 2 ml of benzene—dichloromethane (9:1). After centrifugation the organic layer was transferred into a 5-ml centrifuge tube and evaporated to dryness. The residue was dissolved in 50 μl of ethyl acetate and an aliquot of 3—5 μl of the resulting solution was analyzed by GC—MS with the mass spectrometer set to monitor *m/z* 451 and *m/z* 454 in the GC effluent.

At 30 sec after injection the GC divert valve was turned off and 15 sec later the ionizer was turned on and data collection was begun. The ion ratio of *m/z* 451 to *m/z* 454 in an experimental sample was converted to a concentra-

tion of levorphanol using a calibration curve generated from a linear least squares regression analysis of the *m/z* 451 to *m/z* 454 ion ratios versus amount added data from the analyses of the calibration curve samples. The slope (m) and intercept (b) values from the least squares analysis were used to calculate the amount (x) of levorphanol in an experimental sample given an ion ratio (R) using the equation $x = (R-b)/m$.

RESULTS AND DISCUSSION

The methane negative chemical ionization mass spectra of the pentafluorobenzoyl derivative of levorphanol and levorphanol-d_3 are shown in Fig. 1. As seen in Fig. 1, the M^- molecular anions dominate the spectra. The pentafluorobenzoyl group appears to confer maximum sensitivity for the analysis of phenols by electron-capture GC [10] and for phenolic morphines and morphinans by GC—MS [11].

Selected ion current profiles from the analyses of control plasma spiked with known amounts of levorphanol and levorphanol-d_3 and from experimental plasma from a patient receiving Levo-Dromoran® are shown in Fig. 2.

Based on liquid scintillation measurements of the radioactivity extracted from samples containing tritiated levorphanol, the extraction procedure gave recoveries of the drug from plasma of 86—87%. Calibration curves from the assay are linear, i.e. correlation coefficients are greater than 0.99, for concentrations of levorphanol between 1 and 50 ng ml^{-1} using 10 ng of levorphanol-d_3 as internal standard. The y intercept of the calibration curve using

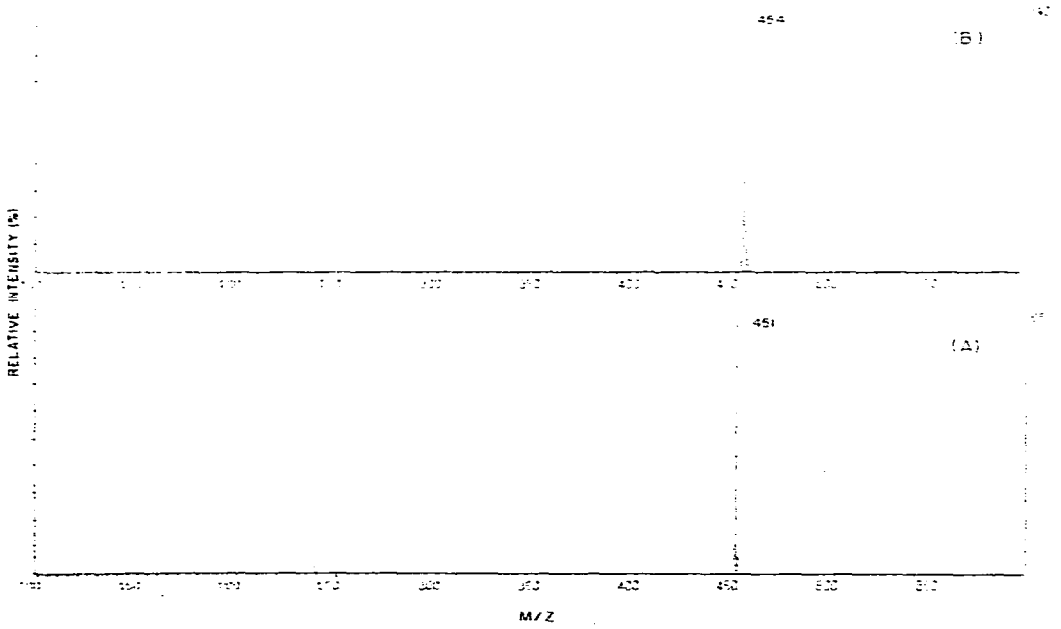

Fig. 1. Methane negative chemical ionization mass spectra of the pentafluorobenzoyl derivatives of (A) levorphanol (mol. wt. = 451) and (B) levorphanol-d_3 (mol. wt. = 454).

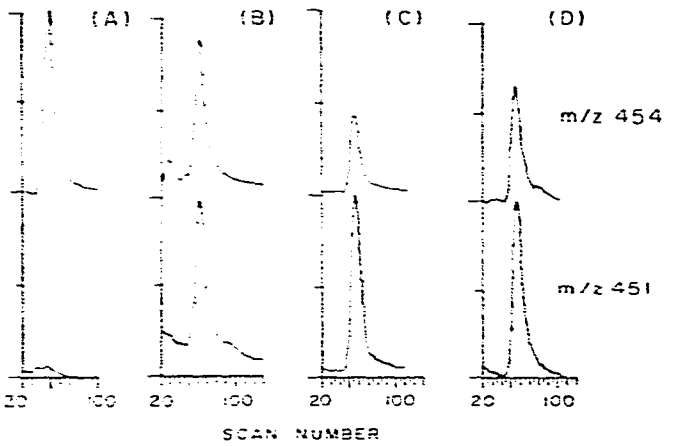

Fig. 2. Selected ion current profiles from the analyses of control plasma (A) and plasma taken from a cancer patient on chronic Levo-Dromoran® therapy just prior to (B), 60 min (C) and 120 min (D) after receiving a 3-mg dose of Levo-Dromoran®. All samples were spiked with 10 ng ml^{-1} of levorphanol-d_3. The concentrations of levorphanol in the samples whose selected ion current profiles are given in (B), (C) and (D) were 10.0, 23.9 and 14.5 ng ml^{-1}, respectively.

TABLE I

CONCENTRATION OF LEVORPHANOL IN PLASMA SAMPLES FROM TEN SUBJECTS ON LEVO-DROMORAN® THERAPY MEASURED BY EITHER THE GC—MS METHOD OR THE RIA PROCEDURE

Sample No.	GC—MS (ng ml^{-1})	RIA (ng ml^{-1}) [5, 6]
1	20	17
2	5	4
3	6	7
4	10	12
5	10	10
6	23	23
7	15	14
8	25	28
9	21	15
10	20	24

10 ng of levorphanol-d_3 as internal standard typically represents less than 0.1 ng of levorphanol. The limit of quantitation is 1 ng ml^{-1}.

The precision of the assay was evaluated by six analyses each of control plasma spiked with either 1, 10 or 50 ng ml^{-1} of levorphanol. The amounts found (± S.D.) were 0.95 ± 0.08, 10.5 ± 0.18 and 50.1 ± 0.17 ng ml^{-1}. These values give the assay a relative standard deviation of 8.2, 1.7 and 0.3% for concentrations of 1, 10 and 50 ng ml^{-1}, respectively.

Comparative analyses of human plasma samples by this GC—MS assay and the published RIA procedure [5, 6] are shown in Table I. A linear regression analysis of the RIA data (y) against the GC—MS data (x) shows them to be

well correlated (correlation coefficient = 0.93). The regression line has a slope of 0.98 and an intercept of only 0.21 ng.

In conclusion, a relatively simple, specific and sensitive assay has been developed to measure levorphanol in human plasma. The assay has confirmed data generated by a published RIA method [5, 6] for levorphanol. The general procedure reported should be useful for the GC–MS analyses of other phenolic morphinans and morphines.

ACKNOWLEDGEMENT

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