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Quantitation of levorphanol in human plasma at subnanogram per milliliter levels using capillary gas chromatography with electron-capture detection

E. Thomas Everhart*, Peter Shwonek, Peyton Jacob III, Michael C. Rowbotham, Reese T. Jones

Drug Dependence Research Center, Department of Psychiatry, University of California at San Francisco, San Francisco, CA 94143, USA

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Abstract

A gas chromatographic method for the determination of levorphanol in human plasma is described. The method utilizes extractive alkylation with tetrabutylammonium cation as the phase-transfer catalyst and pentafluorobenzyl bromide as the alkylating agent, and employs a structural analog, *d*-3-hydroxy-*N*-ethylmorphinan, as the internal standard. The pentafluorobenzyl ethers formed are separated by capillary gas chromatography and detected by electron capture. The method has good precision and accuracy for concentrations ranging from 0.25 ng/ml to 100 ng/ml and has been used to measure plasma concentrations as part of a study to evaluate the management of chronic neuropathic pain with levorphanol.

Keywords: Levorphanol

1. Introduction

Levorphanol, *l*-3-hydroxy-*N*-methylmorphinan, is a synthetic opioid analgesic with a potency, when administered parenterally, of about fivefold that of morphine, and a high oral to parenteral potency ratio [1]. It is used in the management of pain associated with cancer and is currently in clinical trials for the management of chronic neuropathic pain.

For an evaluation of the efficacy of levorphanol for the control of chronic pain, a sensitive analytical method for the measurement of levorphanol in human plasma was required. Dixon et al. [2,3] reported a radioimmunoassay (RIA) procedure capable of measuring concentrations as low as 1 ng/ml, utilizing only 20 μl of plasma. RIA methods are often very sensitive and useful for a variety of clinical applications. This particular method does not appear to suffer from cross-reaction with levorphanol metabolites, because their concentrations in human plasma appear to be insignificant [3]. However, chromatographic methods are generally more specific and may be preferred, particularly for pharmacokinetic studies. The major pathways of levor-

*Corresponding author. Corresponding address: Langley Porter Psychiatric Institute, Box CPR-0984, 401 Parnassus Avenue, San Francisco, CA 94143-0984, USA. Tel.: +1-415-4767-204; fax: +1-415-4767-690.

E-mail address: bluhun@itsa.ucsf.edu (E.T. Everhart)

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phanol metabolism are oxidative *N*-dealkylation to *l*-3-hydroxymorphinan (norlevorphanol) and formation of a glucuronide conjugate [4–6]. These metabolites differ significantly from levorphanol in their physical properties and would not be expected to interfere with a chromatographic assay.

Lucek and Dixon [7] developed a reversed-phase high-performance liquid chromatography (HPLC) method employing electrochemical detection, reporting a limit of quantitation of 1 ng/ml. Several HPLC assays, utilizing ultraviolet [8–10], fluorescence [11–14], and electrochemical [15] detection have been reported for the analysis of dextrorphan, the non-analgesic enantiomer of levorphanol and the major metabolite of the antitussive drug, dextromethorphan. These methods, employed for the determination of debrisoquin-oxidation phenotypes, usually involved determination of urine levels of dextrorphan, and rarely claimed limits of quantitation lower than 25 ng/ml. One method [13] was applied to the analysis of plasma and urine and claimed a limit of detection of 1 ng/ml.

Several published gas chromatographic methods for the measurement of levorphanol in plasma employ packed-column separation of the free phenol from the internal standard and endogenous plasma components, with either flame-ionization (FID) [16], nitrogen–phosphorous (NPD) [17,18] or chemical ionization-mass spectrometric (CI-MS) [18] detection. de Silva et al. [18] claimed sensitivity limits of 10 ng/ml and 2 ng/ml for the NPD and CI-MS methods, respectively. Min et al. [19] published a negative chemical ionization GC–MS method, incorporating deuterium-labeled levorphanol as the internal standard. The limit of quantitation, employing a packed-column separation of pentafluorobenzoyl ester derivatives, was reported to be 1 ng/ml.

Other GC methods, developed for the quantitation of dextrorphan in urine, employed either NPD [20,21] or mass spectrometric [22,23] detection. Kintz et al. [21] reported a limit of quantitation of 0.7 ng/ml.

Based on the doses employed in our clinical studies, it was anticipated that a method with subnanogram per milliliter sensitivity would be required. Previous success in accurately measuring subnanogram per milliliter concentrations of another synthetic opioid, buprenorphine [24], using capillary GC with electron-capture detection (ECD), prompted us to explore this methodology for levorphanol.

In this paper we report a gas chromatographic method with ECD for quantitating levorphanol in plasma. The method involves conversion to the pentafluorobenzyl ether derivative, chromatography on a fused-silica capillary column, and utilizes a structural analog of levorphanol, *d*-3-hydroxy-*N*-ethylmorphinan, as the internal standard. The method is capable of accurately quantitating levorphanol at concentrations as low as 0.25 ng/ml.

2. Experimental

2.1. Reagents and standards

Levorphanol tartrate and desipramine hydrochloride were obtained from Sigma (St. Louis, MO, USA). Dextromethorphan hydrobromide was obtained from ICN Pharmaceuticals (Costa Mesa, CA, USA). Acetic anhydride, ethyl acetate, ethyl ether, methanol, methylene chloride and sodium chloride were Certified ACS grade and obtained from Fisher (Pittsburgh, PA, USA). Ammonium hydroxide and hydrochloric acid were Certified ACS PLUS and obtained from Fisher. Acetonitrile, heptane, 2-propanol, tetrahydrofuran and toluene were HPLC grade and obtained from Fisher. Iodine (USP), perchloric acid, 60–62% (reagent ACS) and sodium sulfate (certified anhydrous) were also obtained from Fisher. Sodium bicarbonate (analytical reagent) and sulfuric acid (analytical reagent) were obtained from Mallinkrodt (St. Louis, MO, USA). Sodium hydroxide (10 *M*) (reagent grade) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Boron tribromide (1.0 *M* solution in dichloromethane), α-bromo-2,3,4,5,6-pentafluorotoluene, 99+%, borane–tetrahydrofuran (THF) complex (1.0 *M* solution in tetrahydrofuran), calcium sulfate, anhydrous (Drierite), 1-chloroethyl chloroformate, 98%, activated carbon (Norit) and tetrabutylammonium dihydrogenphosphate, 97%, were obtained from Aldrich (Milwaukee, WI, USA). Aquasil water-soluble siliconizing fluid was obtained from Pierce (Rockford, IL, USA). Thin-layer chromatography (TLC) plates were pre-scored, 20×10 cm, silica gel GF Uniplates (250 μ layer thickness) purchased from Analtech (Newark, DE, USA). The

internal standard, *d*-3-hydroxy-*N*-ethylmorphinan, was synthesized as described below.

2.2. Synthesis of the internal standard

2.2.1. d-3-methoxymorphinan

A solution of dextromethorphan free base (3.75 g, 13.8 mmol) and 1-chloroethyl chloroformate (3 ml, 3.96 g, 27.7 mmol) in 70 ml of toluene was protected from atmospheric moisture and heated under reflux overnight. The mixture was cooled to room temperature, the solvent removed under reduced pressure and the intermediate chlorocarbamate decomposed by refluxing in 100 ml of methanol for 2.5 h. Removal of the solvent under reduced pressure and dissolution of the brown, oily residue into 150 ml of water was followed by adjustment of the pH to 12 with sodium hydroxide and extraction of the liberated free base into 125 ml of dichloromethane. The aqueous phase was extracted a second time with 125 ml of dichloromethane, and the combined extracts were washed with 125 ml of saturated sodium chloride, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield 3.45 g of a dark red oil. Purification by flash chromatography on 200–400 mesh silica gel, employing acetonitrile–methanol–ammonia (6:4:1, v/v/v) as the mobile phase, yielded 3.05 g (11.9 mmol, 86.2%) of pale amber *d*-3-methoxymorphinan. TLC on silica gel, employing acetonitrile–methanol–ammonia (6:4:1, v/v/v) as the mobile phase, exhibited a single spot at R_F 0.28.

2.2.2. d-3-methoxy-N-acetylmorphinan

The acetylation of *d*-3-methoxymorphinan was accomplished by dissolving 1.80 g (7.00 mmol) of material in 100 ml of acetic anhydride–pyridine (1:1, v/v) and stirring at room temperature overnight. Removal of the excess reagent under reduced pressure gave an orange oil which was dissolved in 100 ml of ethyl acetate, washed with 1 *M* HCl (4×100 ml) and 100 ml of saturated sodium chloride, dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure to yield 1.90 g (6.35 mmol, 90.7%) of a viscous, pale yellow oil. TLC, employing the same solvent system above, exhibited a single spot at R_F 0.83. Analysis by gas chromatography–mass spectrometry (GC–MS) indicated a single peak with a spectrum consistent with the structure: *m*/*z* (relative intensity) 299 (molecular ion, 6), 213 (26), 171 (7), 87 (100), 72 (30), 43 (CH_3CO^+, 6).

2.2.3. d-3-methoxy-N-ethylmorphinan

To 15 ml of a 1 *M* solution of borane–tetrahydrofuran complex in THF, maintained at 0°C and protected from atmospheric moisture, was added a solution of 1.90 g (6.35 mmol) of *d*-3-methoxy-*N*-acetylmorphinan in 25 ml of dry tetrahydrofuran. The stirred reaction mixture was warmed to room temperature and heated at reflux for 1 h. After cooling to room temperature, the excess reagent was decomposed by the slow addition of 20 ml of 3 *M* HCl, and tetrahydrofuran removed by distillation at atmospheric pressure. The aqueous residue was extracted with ethyl ether (3×30 ml), basified with 10 *M* NaOH, and again extracted with ether (3×20 ml). The combined basic extracts were dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give 1.80 g (6.32 mmol, 99.5%) of a pale yellow oil. GC–MS exhibited a single peak with the mass spectrum in accordance with the structure: *m*/*z* (relative intensity) 285 (molecular ion, 27), 270 ($M-CH_3$, 100), 214 (10), 171 (15), 164 (13), 135 (10), 73 (15), 58 (20), 44 (19).

2.2.4. d-3-hydroxy-N-ethylmorphinan perchlorate

To 16.5 ml of a stirred 1 *M* solution of boron tribromide in dichloromethane, protected from atmospheric moisture, was slowly added a solution of 1.50 g (5.27 mmol) of *d*-3-methoxy-*N*-ethylmorphinan in 15 ml of dichloromethane. The reaction mixture was allowed to stir overnight and was quenched by the slow addition of 25 ml of 3 *M* ammonium hydroxide. The dichloromethane was removed by distillation at atmospheric pressure, and the two-phase mixture, consisting of brown, gummy residue and aqueous ammonia, was extracted with 40 ml of ethyl ether. The aqueous layer was re-extracted with ether (2×40 ml), and the combined extracts were washed with 50 ml of saturated sodium chloride, dried over anhydrous sodium sulfate and filtered. After decolorizing the red solution with activated carbon (Norit) and filtering from the suspended solids, evaporation under reduced pressure yielded 1.27 g (4.69 mmol, 89%) of a foamy white

solid. The solid was dissolved in 110 ml of 2-propanol–ether (10:1, v/v) and treated with 0.6 ml of 60–62% perchloric acid. The precipitated perchlorate salt was filtered from solution, washed first with fresh 2-propanol–ether (10:1, v/v), then with ethyl ether, and air dried to give 1.55 g of an off-white crystalline powder. Recrystallization from 2-propanol–methanol (5:1, v/v) yielded 0.96 g (2.6 mmol, 49% from *d*-3-methoxy-*N*-ethylmorphinan) of fine white needles, melting with decomposition at 261–262°C. TLC on silica gel, employing acetonitrile–methanol–ammonia (6:4:1, v/v/v) as the developing solvent, exhibited a single spot at R_F 0.74. The following elemental analysis was calculated for $C_{18}H_{26}NClO_5$: C, 58.14; H, 7.05; N, 3.77; Cl, 9.53; O, 21.51. The results found were: C, 58.21; H, 7.29; N, 3.74; Cl, 9.39; O, 21.61.

2.3. Extraction and derivatization procedure

To 1.0 ml of sample in a silanized 100×16 mm screw-top culture tube were added 50 μl of a 1 μg/ml solution of internal standard (I.S.) [*d*-3-hydroxy-*N*-ethylmorphinan] in 0.01 *M* sulfuric acid, 5 ml of toluene and 0.5 ml of pH 9.5, 10% sodium carbonate buffer. The tube was then capped, vortex-mixed for 5 min, centrifuged for ca. 5 min at 1500 *g* and subsequently immersed in a dry ice–acetone bath to freeze the bottom, aqueous layer. The top organic layer was decanted into a clean 100×16 mm culture tube containing 200 μl of 0.1 *M* H_2SO_4. This tube was capped, vortex-mixed for 5 min, centrifuged for 5 min, frozen in dry ice–acetone and the toluene was discarded. To the aqueous phase remaining in each tube was added 2 ml of heptane, and the tube was re-capped, vortex-mixed for 1 min, centrifuged for 5 min, frozen in dry ice–acetone and the organic layer was discarded. The derivatization was accomplished by the addition of 0.5 ml of 0.1 *M* tetrabutylammonium dihydrogenphosphate, 100 μl of 10 *M* NaOH, 200 μl of 0.4% (v/v) pentafluorobenzyl bromide in dichloromethane and vortex-mixing at room temperature for 30 min. A 2 ml-volume of heptane was added to each tube, which was then vortex-mixed for 10 s, centrifuged for 5 min, and frozen in dry ice–acetone.

The organic layer was poured into a third tube containing 0.5 ml of 0.5 *M* sulfuric acid. This mixture was vortex-mixed for 1 min, centrifuged for 5 min, frozen in dry ice–acetone and the organic layer discarded. The aqueous phase was washed with an additional 2 ml of heptane by repetition of the above procedure. Finally, 120 μl of 5 *M* NaOH and 2 ml of heptane were added to the tube, which was vortex-mixed for 5 min and centrifuged for 5 min. The aqueous phase was frozen in dry ice–acetone and the organic layer decanted into a clean 100×16 mm culture tube. Evaporation of the solvent under a stream of nitrogen was followed by reconstitution of the residue in 50 μl of 4 mg/ml solution of desipramine in *n*-octane. The solutions were transferred to autosampler vials with silanized 200-μl microvial inserts, crimp-capped and loaded onto the autosampler tray. A 2-μl volume was injected into the gas chromatograph.

2.4. Gas chromatography

GC analyses were performed using a Hewlett-Packard (HP) 5890 GC equipped with an HP 7673A autosampler, an HP G1223A electron-capture detector, and an HP G2071AA data collection and processing system (Hewlett-Packard, Palo Alto, CA, USA). A Restek Rtx-65TG fused-silica capillary column (30 m×0.25 mm I.D.) was used, with a stationary phase of cross-linked 65% diphenyl-, 35% dimethyl-silicone gum (0.10 μm film thickness). A high-pressure Merlin Microseal (Merlin Instruments, Half Moon Bay, CA, USA) was used instead of a standard silicone-rubber septum. Helium (99.999%) was utilized as the carrier gas, with a head pressure of 150 kPa, which resulted in a flow-rate of approximately 2.25 ml/min (28 cm/s) at 150°C.

The sample (2 μl) was injected in the splitless mode via the autosampler, with a septum purge on-time of 1 min and an injection-port temperature of 285°C. The column oven temperature was programmed from 150°C (after a 1 min hold) to 310°C at a rate of 10°C/min. The detector temperature was 325°C. Typical retention times for the pentafluorobenzyl derivatives were 13.67 min (levorphanol) and 13.83 min (I.S.).

2.5. Mass spectroscopy

Mass spectroscopy data was obtained using a Hewlett-Packard 5890 Series II GC with an HP 7673 autosampler, an HP 5971A mass-selective detector,

and an HP G1034C MS data collection and processing system. Analyses were performed using a Restek Rtx-1 fused-silica capillary column (30 m×0.25 mm I.D.) with a stationary phase of cross-linked methylsilicone gum (0.50 μm film thickness). Helium was used as the carrier gas with a head pressure of 100 kPa, which resulted in a flow-rate of approximately 2.3 ml/min (19 cm/s) at 150°C. The oven-temperature program was the same as that utilized for the GC–ECD analyses. The mass spectrometer was operated in the electron-ionization mode. Data were collected in the scan mode (50–550 u). The mass spectra of the pentafluorobenzyl (PFB) ether derivatives were consistent with the structures. Levorphanol, PFB ether: *m/z* (relative intensity) 437 (molecular ion, 2.6), 256 (53), 181 ($C_7F_5H_2^+$, 100), 150 (26), 128 (13); *d*-3-hydroxy-*N*-ethylmorphinan, PFB ether: *m/z* (relative intensity) 451 (molecular ion, 1.2), 436 ($M-CH_3$, 7), 270 (20), 181 ($C_7F_5H_2^+$, 100), 128 (11).

2.6. Quantitation

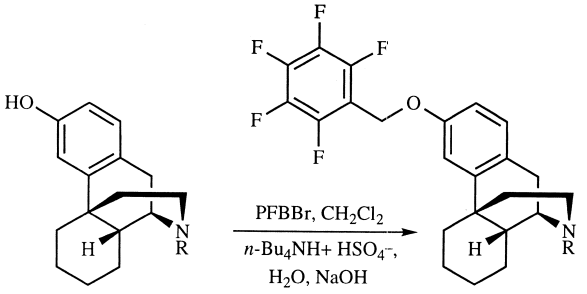
Standards and controls were prepared from appropriately diluted aliquots of stock solutions containing 100 μg/ml of levorphanol in 0.01 *M* H_2SO_4, spiked into blank plasma to achieve a concentration of 100 ng/ml. Twelve plasma standards, spanning the range of 0.25 to 100 ng/ml, were prepared by diluting the 100 ng/ml standard with the appropriate volume of blank plasma. The concentrations of the calibrators were 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 5.00, 7.50, 10.0, 25.0, 50.0 and 100 ng/ml. Six plasma controls, spanning the range from 0.25 to 50 ng/ml, were prepared in the same manner. Their concentrations were 0.25, 0.50, 2.00, 10.0, 25.0 and 50.0 ng/ml. Quantitation was achieved by integration of detector responses and constructing standard curves of the response (peak-height) ratios of the analyte/internal standard versus amount (concentration) ratios of analyte/internal standard by nonlinear regression. The controls were used in duplicate for all sample runs to assess run quality. Standard curves were broken into low and high levels. The low level ranged from 0.25 to between 2 and 5 ng/ml. The high level ranged from between 0.25 and 100 ng/ml. The cut-off was established during data processing for each run.

2.7. Repeatability, precision and accuracy

The repeatability of the method was estimated by comparing the second-order regression equations, intercepts and correlation coefficients of the calibrator plots from individual runs. Precision and accuracy were determined from the analysis of spiked plasma controls at six concentrations ranging from 0.25–50 ng/ml. The precision of the method was expressed as the intra- and inter-day relative standard deviations (RSDs), and the accuracy as the ratio of the average calculated concentrations to their spiked values.

3. Results and discussion

Conversion of levorphanol to the pentafluorobenzyl ether derivative (Fig. 1) was carried out to provide high sensitivity with an electron capture detector and to improve chromatographic properties by masking the polar phenolic hydroxy group. This derivative had been previously evaluated by de Silva et al. [18] in the development of a chemical ionization method for the measurement of levorphanol in plasma, but was rejected because of extensive fragmentation of the protonated molecular ion in the methane–ammonia chemical ionization (CI) spectrum. A previously described method for GC–ECD determination of the structurally related drug pentazocine [25,26] was modified for extraction and derivatization of levorphanol. The method employed



Ia, R = Me
b, R = Et

IIa, R = Me
b, R = Et

Fig. 1. Conversion of levorphanol and the internal standard (*d*-3-hydroxy-*N*-ethylmorphinan) to the pentafluorobenzyl ether derivatives.

tetra-*n*-butylammonium cation as the phase-transfer catalyst in an extractive-alkylation procedure to form the pentafluorobenzyl ether derivative, and GC determination using a packed column. We optimized the initial extraction procedure to maximize the recovery of levorphanol, utilized a structural analog of levorphanol as the internal standard to maximize precision and accuracy, and employed modern capillary GC to enhance sensitivity and facilitate separation from endogenous substances.

Conversion of levorphanol to the pentafluorobenzyl ether was readily accomplished by extractive alkylation employing pentafluorobenzyl bromide as the alkylating agent and tetra *n*-butylammonium cation as the phase-transfer catalyst (Fig. 1). The resulting PFB ether exhibited excellent chromatographic properties, particularly at subnanogram per ml concentrations. Fig. 2 illustrates a chromatogram of an extracted and derivatized 1-ml blank plasma sample supplemented with 5 ng levorphanol and 50 ng of the internal standard, the structural analog *d*-3-hydroxy-*N*-ethylmorphinan. Fig. 3 is an expanded chromatogram of a 1-ml blank plasma sample spiked with 0.5 ng of levorphanol and 50 ng of the I.S., and Fig. 4 is an expanded chromatogram of an unspiked blank plasma sample. The advantages of using internal standards that are close structural analogs of analytes are well documented. The internal standard was extracted and derivatized in a fashion analogous to the analyte, and effectively compensated for losses of the drug during the several extraction and back-extraction steps required for the elimination of endogenous plasma components and co-administered drugs and their metabolites.

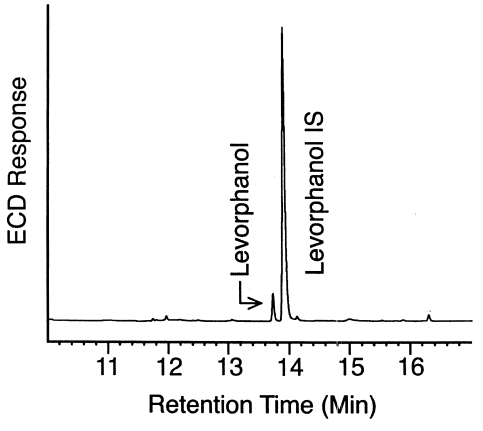


Fig. 2. Chromatogram of an extracted, derivatized 1 ml blank plasma sample supplemented with 5 ng of levorphanol and 50 ng of the internal standard (*d*-3-hydroxy-*N*-ethylmorphinan).

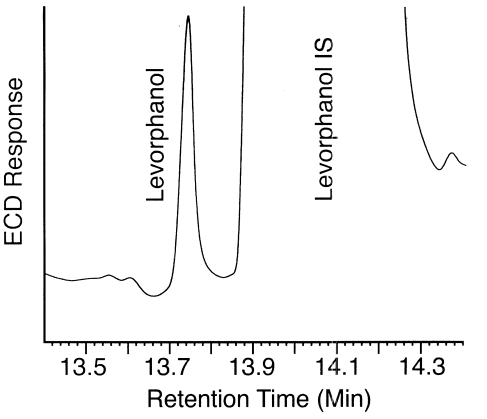


Fig. 3. Expanded chromatogram of an extracted, derivatized 1 ml blank plasma sample supplemented with 0.5 ng of levorphanol and 50 ng of the internal standard (*d*-3-hydroxy-*N*-ethylmorphinan).

Interference from residual co-extracted and derivatized endogenous plasma components necessitated the use of a thin-film, highly phenyl-substituted Rtx-65TG column. Adsorption of the pentafluorobenzyl ether derivatives and carryover from chromatogram to chromatogram represented a significant problem, compromising the ability to quantitate levorphanol at subnanogram per ml levels in plasma. The problem was substantially reduced by dissolving the evaporated, derivatized extracts in a dilute solution of

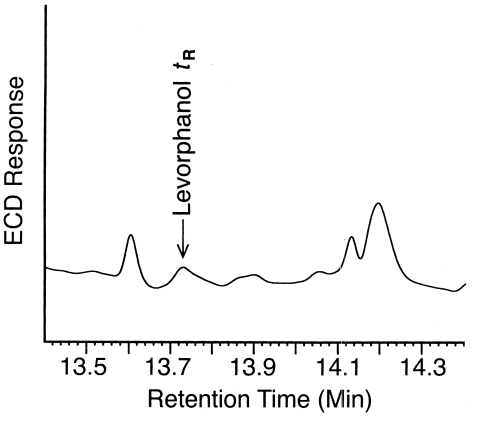


Fig. 4. Expanded chromatogram of an extracted, derivatized, nonsupplemented 1 ml blank plasma sample.

Table 1
Equations for standard curves, *O*-pentafluorobenzyl derivatives[a]

Analyte	Concentration range (ng/ml)	*a*	*b*	Intercept	Coefficient of determination (r^2)
Levorphanol	0.25 to 2.00–5.00	5.003±2.077	0.4671±0.1061	0.00128±0.00039	0.99932±0.00067
	0.25 to 50–100	−0.0671±0.0260	1.067±0.129	−0.0086±0.0045	0.99986±0.00006

[a] Equations were determined by nonlinear regression: response ratio=$a\times$(amount ratio)$^2+b\times$amount ratio+intercept.

desipramine free base in *n*-octane. The desipramine functioned as an adsorption inhibitor and possessed a negligible electron-capture detector response [25,26]. The *n*-octane, significantly less volatile than the *n*-heptane or *n*-hexane employed in analogous methods for pentazocine [25,26], resulted in significantly less evaporation. When heptane was employed, evaporation caused separation of desipramine from solution, resulting in plugged syringe needles and seized syringe plungers. Silanization of the culture tubes employed for extraction and derivatization, as well as the GC-microvial inserts, also inhibited losses of analytes and derivatives by adsorption.

The calibration curves were broken into high and low levels, with the low level running from 0.25 to between 2 and 5 ng/ml, and the high level ranging from 0.25 and 100 ng/ml. Cut-offs were established as part of the data analysis procedure after completion of each run. The upper limit of the low-level curve was determined by applying nonlinear least-squares regression analysis to all data points between 0.25 and 5 ng/ml. Points were sequentially eliminated from the high end of the curve until the best fit was obtained. The curve was represented by a second-order polynomial function. The high-level curve could be approximated by a linear function, but we chose to represent it with a second-order polynomial to improve the precision and accuracy of the calculated quality-control sample concentrations (Table 1).

There was essentially no interference from endogenous compounds when the Rtx-65TG column was employed, allowing accurate quantitation down to 0.25 ng/ml. Precision and accuracy were excellent at each level. Intra-day precision (RSD) and accuracy (percent of actual concentration) ranged from 3.1 to 10.5%, and 92.4 to 114%, respectively (Table 2). Inter-day precision and accuracy ranged from 5.0 to 13.5%, and 96.4 to 103%, respectively (Table 3). The limit of quantitation was defined as the lowest concentration that could be measured with an accuracy of within 15% of the actual concentration, and with an inter-day precision of 15% or less.

To evaluate the possibility of chromatographic interference from other drugs administered to the patients participating in the clinical study, we obtained a list of the 91 total co-medications and investigated each substance individually. The drugs either did not extract from the plasma under the conditions employed in the assay, did not back-extract into acid, did not form an electron-capturing derivative, or formed a nonvolatile derivative. No interferences were found. The data is summarized in Table 4.

Norlevorphanol, the *N*-dealkylated metabolite of levorphanol, was extracted and derivatized. How-

Table 2
Intra-day precision and accuracy for the determination of levorphanol in plasma

Actual concentration (ng/ml)	Measured mean (ng/ml)	Accuracy (%)	RSD (%)	Replicate analyses
0.25	0.231	92.4	7.7	7
0.50	0.474	94.8	10.5	8
2.00	2.27	114	3.1	8
10.0	10.49	105	6.9	8
25.0	26.23	105	6.0	8
50.0	50.94	102	5.9	8

Table 3
Inter-day precision and accuracy for the determination of levorphanol in plasma

Actual concentration (ng/ml)	Measured mean (ng/ml)	Accuracy (%)	RSD (%)	Replicate analyses
0.25	0.244	97.6	13.5	10
0.50	0.509	102	7.7	10
2.00	2.04	102	9.1	10
10.0	9.64	96.4	5.0	10
25.0	24.89	99.6	5.5	10
50.0	51.61	103	6.6	10

ever, the derivative eluted approximately 0.4 min after the internal standard and did not interfere with the assay.

In patients taking levorphanol for the alleviation of chronic neuropathic pain and averaging daily oral doses of from 1.2 to 15.75 mg levorphanol over a period of two weeks, measured plasma concentrations ranged from 0.33 to 10.5 ng/ml. The clinical data are currently being evaluated and will be the subject of a separate publication.

In summary, a sensitive GC method has been developed for the determination of levorphanol in human plasma. The method has good precision and accuracy in the concentration ranges typically found

Table 4
Co-medications administered to subjects in the clinical study

Acetaminophen[b]	Codeine[c]	Hydroxyzine[c]	Nitrofurantoin[c]
Acyclovir[c]	Cyclobenzaprine[c]	Ibuprofen[a]	Nortriptyline[c]
Albuterol[d]	Desipramine[c]	Imipramine[c]	Oxybutynin[c]
Allopurinol[d]	Dexpanthenol[a]	Ipratropium[a]	Pentoxifylline[c]
Alprazolam[c]	Diazepam[c]	Ketoprofen[a]	Phenytoin[b]
Althiazide[d]	Diclofenac[a]	Lamotrigine[c]	Pyridostygmine[a]
Amantidine[c]	Digoxin[a]	Levothyroxine[a]	Quinidine[c]
Amiloride[c]	Diphenhydramine[c]	Lidocaine[c]	Quinine[c]
Amitriptyline[c]	Doxazosin[c]	Lisuride[c]	Ranitidine[c]
p-Aminosalicylic acid[a]	Doxycycline[a]	Loperamide[c]	Sertraline[c]
Amoxicillin[a]	Ergonovine[c]	Lorazepam[c]	Simvastatin[b]
Apraclonidine[c]	Estradiol[b]	Lovastatin[b]	Spironolactone[b]
Aspirin[a]	Estrone[b]	Medroxyprogesterone[b]	Sulfamerazine[d]
Baclofen[a]	Etodolac[a]	Melatonin[c]	Sumatriptan[d]
Beclamethasone[b]	Famotidine[d]	Mesalamine[a]	Temazepam[c]
Bupropion[c]	Fluoxetine[c]	Metaclopramide[c]	Theophylline[c]
Capsaicin[b]	Flurazepam[c]	Metformin[c]	Timolol[c]
Captopril[a]	Furosemide[a]	Mexiletine[c]	Trazodone[c]
Carbemazepine[b]	Glipizide[d]	Minocycline[a]	Triamcinolone[b]
Chlorpheniramine[c]	Glyburide[b]	Naproxen[a]	Triamterene[c]
Cimetidine[c]	Guaifenesin[b]	Nicardipine[c]	Verapamil[c]
Ciprofloxacin[a]	Hydrocodone[c]	Nicotinic acid[a]	Warfarin[b]
Clonazepam[c]	Hydrocortisone[b]	Nifedipine[c]	

[a] Does not extract from plasma under conditions of the assay.
[b] Does not back-extract into acid.
[c] Does not form electron-capturing derivative.
[d] Forms non-volatile derivative (no peak observed).

in plasma, and has been used to monitor the levorphanol blood levels of human subjects participating in clinical trials of levorphanol for pain management.

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