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Journal of Chromatography B, 775 (2002) 57–62

JOURNAL OF CHROMATOGRAPHY B

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Validation and application of a sensitive assay for butorphanol in human plasma by high-performance liquid chromatography with tandem mass spectrometry detection

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Received 14 January 2002; received in revised form 24 April 2002; accepted 24 April 2002

Abstract

A sensitive and convenient high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the opioid receptor agonist–antagonist butorphanol in human plasma is described. BC-2605, a cyclopropyl analogue of butorphanol, was employed as an internal standard. Butorphanol was recovered from plasma (84.4±10.9%) by liquid–liquid extraction. The mobile phase flow-rate was 0.3 ml/min and consisted of methanol–water–formic acid (90:10:0.1, v/v/v). The analytical column (4.6×100 mm) was packed with Partisil C_8 (5 μm). The standard curve was linear from 13.7 to 1374 pg/ml (r^2>0.99). The lower limit of quantitation was 13.7 pg/ml. The assay was specific, accurate (% deviation from nominal concentrations were <15%), precise and reproducible (within- and between-day coefficients of variation <7%). Butorphanol in plasma was stable over 3 freeze/thaw cycles and at room temperature for 1 day. The utility of the assay was demonstrated by following butorphanol plasma concentrations in two healthy subjects for 24 h following a 1 mg intranasal dose.

Keywords: Butorphanol

1. Introduction

Butorphanol tartrate [(−)-17-(cyclobutylmethyl)morphinan-3,14-diol hydrogen tartrate, see Fig. 1] is a μ-opioid receptor agonist/antagonist and is an effective analgesic used for the relief of moderate to severe pain [1]. Butorphanol tartrate is available as a nasal spray formulation, normally taken as a 1 or 2 mg dose, which provides rapid relief of pain symptoms lasting for 5–6 h [2]. This formulation has proved effective in managing pain such as that suffered in migraine attacks or severe headaches [3] and has been used in cases of dental, maxillofacial or other surgical pain [4,5].

Plasma concentrations of intranasally administered butorphanol following a single dose or at steady-state following repeated regular dosing are relatively low, typically being less than 5 ng/ml at normal doses [6,7]. Therefore, a relatively sensitive picogram-level assay is required to evaluate the pharmacokinetics of

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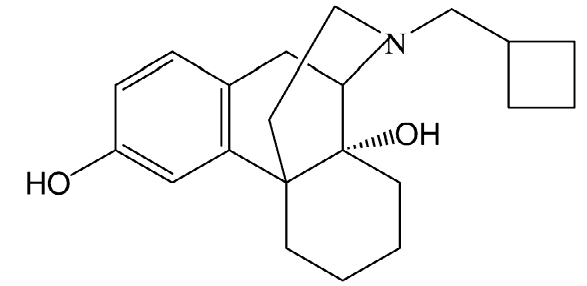
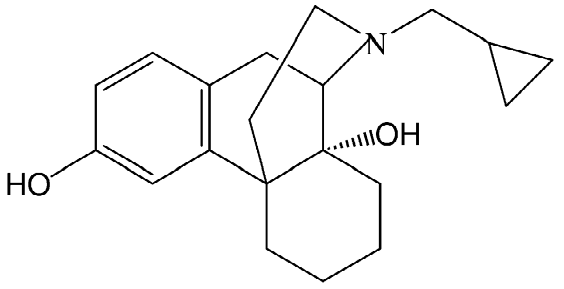
1570-0232/02/$ – see front matter
PII: S1570-0232(02)00277-5



Butorpanol



BC-2605

Fig. 1. Molecular structures of butorphanol and the internal standard, BC-2605.

butorphanol. Assay methods for butorphanol in clinical plasma samples that have been reported to date have included radioimmunoassay (RIA) [8] and gas chromatography (GC) with mass spectrometry (MS) detection [9]. While both of these methods are sensitive enough to quantitate butorphanol, the handling of radioactivity and the need for multiple standard curves are disadvantages of RIA, while the necessity for pre-analysis sample derivatization is a disadvantage of GC–MS; thus, a simpler analytical methodology was desirable. This report describes the development and validation of a convenient and sensitive high-performance liquid chromatography (HPLC) method with tandem MS (MS/MS) detection of butorphanol in human plasma and its application to the analysis of clinical samples.

2. Materials and methods

2.1. Materials

Butorphanol tartrate reference standard (100% pure, concentrations are hereafter reported as the base, unless otherwise stated) was obtained from United States Pharmacopeia (Rockville, MD, USA). BC-2605, a cyclopropyl analogue of butorphanol (see Fig. 1), was employed as an internal standard and was synthesized by Bristol-Myers Squibb Co. (Syracuse, NY, USA). Drug-free human plasma, containing EDTA as an anticoagulant, was obtained from the Metropolitan-Washington Blood Bank (Washington, DC, USA). HPLC grade methanol was obtained from Burdick and Jackson (Muskegon, MI, USA). Formic acid, methyl *t*-butyl ether, and ammonium hydroxide, all ACS grade, were obtained from EM Science (Cincinnati, OH, USA). ACS grade ammonium phosphate was obtained from Mallinkrodt (Paris, KY, USA). Water was distilled and deionized by reverse osmosis and subsequently filtered through a 0.45 μm filter.

2.2. Chromatography and mass spectrometry

The high-performance liquid chromatography was performed on a Waters 2690 separations module (combined pump and autosampler, Waters Corp., Milford, MA). The mobile phase flow-rate was 0.3 ml/min and consisted of methanol–water–formic acid (90:10:0.1, v/v/v) and was degassed under vacuum and filtered (0.45 μm) prior to use. The injection volume was 20 μl. The analytical column was a 10 cm×4.6 mm I.D. Partisil C_8, 5 μm particle size (Whatman, Clifton, NJ). The chromatography was performed at ambient temperature. The mass spectrometer was an API Sciex Model 300 (Foster City, CA, USA). The ion polarity was set in positive mode, and the source was TurboIon spray. The nebulizer gas was air (zero grade) and the auxiliary, curtain and collision gas was nitrogen (99.999%). The heated capillary temperature was 400 °C. The ion spray, orifice, and ring voltages were 4800, 50, and 360 V, respectively. The Q0, Q1, Q2, and Q3 rod offset voltages were −5.5, −10, −37.5, −50 V, respectively. The MS/MS transition monitored for butorphanol was at 328.2 to 310.1 amu and for

BC-2605 was at 314.2 to 296.2 amu. Mass spectrometry data were acquired by the system software supplied by PE Sciex (Sample Control v1.2). Chromatographic data were integrated using MacQuan software v1.4 (Applied Biosystems, Foster City, CA, USA).

2.3. Sample preparation

In a 15 ml glass centrifuge tube, 20 μl of the internal standard spiking solution (containing 100 pg of BC-2605) was added to 1 ml of the plasma sample to be analyzed. After vortexing the mixture for 30 s, 1 ml of 1 *M* ammonium phosphate (adjusted to pH 8.6 with ammonium hydroxide) was added to the tube. Subsequently, 5 ml of methyl *t*-butyl ether was added to the mixture, and the tube was vortexed for 5 min, followed by an additional 10 min mixing on a mechanical shaker. The tube was then centrifuged for 10 min at 1200 *g*. The aqueous layer was frozen by placing the tube in a dry ice–acetone bath, and the organic layer was transferred to a clean glass centrifuge tube. The organic solvent was evaporated off in a multicap concentrator under vacuum. The residue was reconstituted in 200 μl of methanol and the mixture transferred into autosampler vials for analysis.

2.4. Quantification and validation

Butorphanol tartrate stock solutions (100 μg/ml) were prepared in methanol and serially diluted to produce a 4000 ng/ml (as tartrate) stock solution. The stock solution was used to prepare various concentrations of spiking solutions in water (0.4–40 ng/ml as tartrate). These spiking solutions were added to drug-free plasma (50 μl of spiking solution to 1 ml of plasma) to produce standard curve and quality control standards. A 5 ng/ml in water spiking solution of the internal standard, BC-2605, was similarly prepared. All stock and spiking solutions were stored at 4±2 °C and used within 1 month of preparation.

The recovery of butorphanol from the extraction procedure (Section 2.3) from human plasma was determined by a comparison of the peak area of butorphanol in spiked plasma samples in triplicate at 34.4, 137 and 687 pg/ml to the peak area of butorphanol in samples prepared by spiking extracted drug-free plasma samples with the same amounts of butorphanol at the step immediately prior to chromatography. The specificity of the extraction procedure and the assay was examined by extracting and analyzing drug-free plasma from six different individuals. The resultant ion chromatograms were examined for the presence of any endogenous constituents which may potentially interfere with the analysis of butorphanol or the internal standard.

Triplicate seven point standard curves ranging from 13.7 to 1374 pg/ml of butorphanol were run on three separate days. The integrated ion chromatogram peak areas of butorphanol and BC-2605 were used to construct a standard curve from the peak area ratio versus nominal butorphanol concentration using linear regression analysis with $1/x$ weighting. Four replicates of quality control standards at three concentrations of butorphanol (41.2, 206 and 1031 pg/ml) were included in each run to determine the within-and between-run precisions of the assay. The lower limit of quantitation (LLQ) was assessed by analyzing plasma samples from the six different individuals spiked with 13.7 pg/ml of butorphanol, the lowest concentration on the standard curve.

The stability of butorphanol in human plasma was assessed by analyzing triplicate quality control samples at 41.2, 206, and 1031 pg/ml stored for 1 day at room temperature and also following one, two, or three cycles of freezing at −70 °C and thawing. Concentrations following storage were compared to freshly-prepared samples of the same concentrations.

2.5. Single intranasal dose pharmacokinetic study

Two healthy young male subjects participated in a pharmacokinetic study. Each subject received a 1 mg intranasal dose of butorphanol tartrate nasal spray (Stadol NS 7®, Bristol-Myers Squibb Co., Princeton, NJ). Serial blood samples were collected into vacuum tubes (containing EDTA) immediately before dosing and at 5, 10, 15, 20, 30, 35, 40, 45 min, and at 1, 1.25, 1.5, 2, 2.5, 4, 6, 8, 12 and 24 h after dosing. Within 1 h of collection, plasma was harvested from the blood samples following centrifugation for 15 min at 2000 *g*. The plasma was immediately frozen and remained frozen at −20 °C until analyzed. The Western Institutional Review Board (Olympia, WA) approved the protocol and informed

consent and the subjects gave written informed consent to participate.

3. Results and discussion

3.1. Chromatography/mass spectrometry

The mass spectrum and product ion mass spectrum of butorphanol are shown in Fig. 2. Ion chromatograms for an extracted drug-free plasma sample and a plasma sample spiked with 13.7 pg/ml of butorphanol (the LLQ) are shown in Fig. 3.

3.2. Quantification and validation

The mean recoveries of butorphanol from human plasma following the extraction procedure were 93.2±9.0, 84.3±11.1 and 75.7±6.6% at 34.4, 137, and 687 pg/ml, respectively. The mean overall recovery of butorphanol was 84.4±10.9% ($n=9$). No significant peaks interfering with butorphanol or the internal standard were observed in drug-free plasma samples from six different individuals (see Fig. 3a).

The correlation coefficients for the standard curves (area ratio = concentration × slope + intercept) ranged from 0.994 to 0.996, and the mean (SD) slope and intercept for the regression lines of best fit were 0.00828 (0.0008) and 0.141 (0.084) ml/pg, respectively ($n=3$). The coefficient of variation (SD/mean) of the regression slopes was 9.9% and the *y*-intercept was not significantly different from zero (2-sided Student's *t*-test). The mean absolute deviations of the predicted compared to nominal butorphanol concentration in LLQ samples was 15.0% with a coeffi-

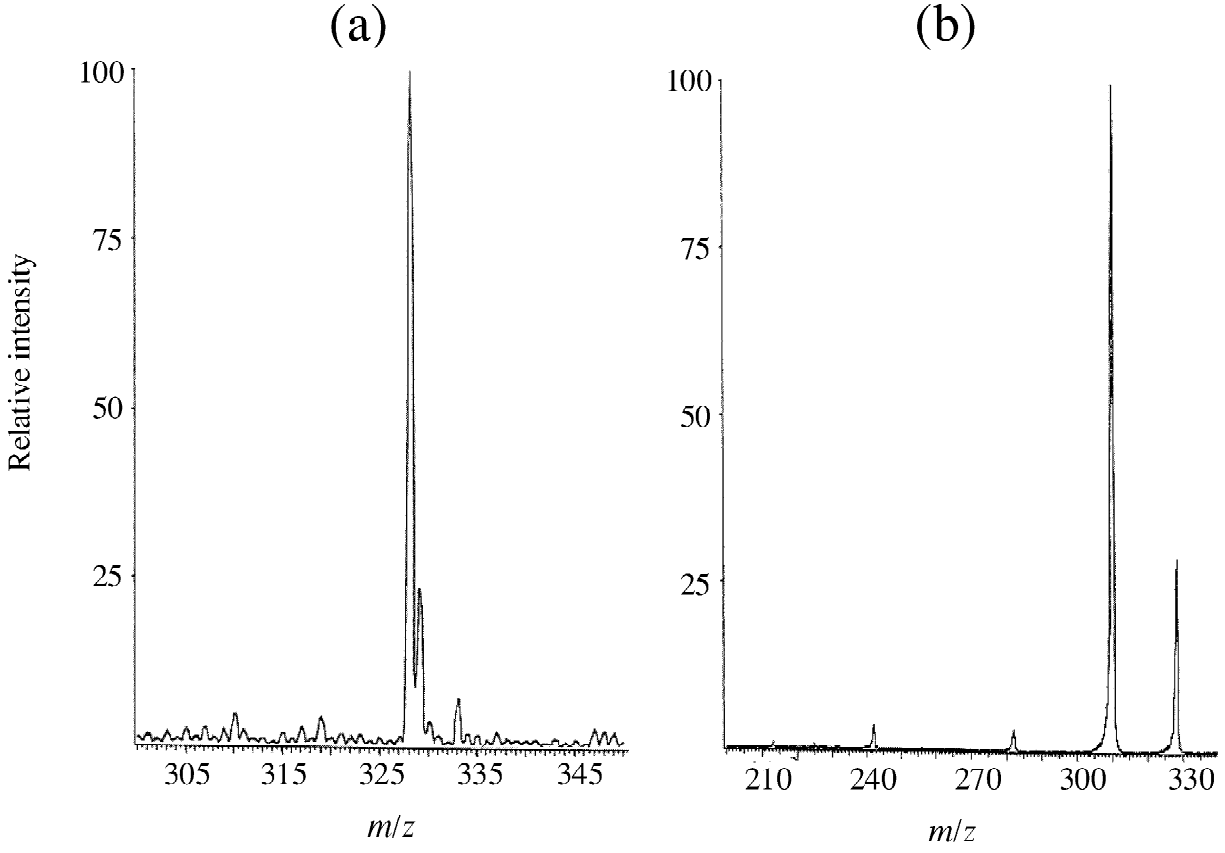


Fig. 2. (a) The mass spectrum of butorphanol and (b) the product ion mass spectrum of butorphanol (m/z 328.2).

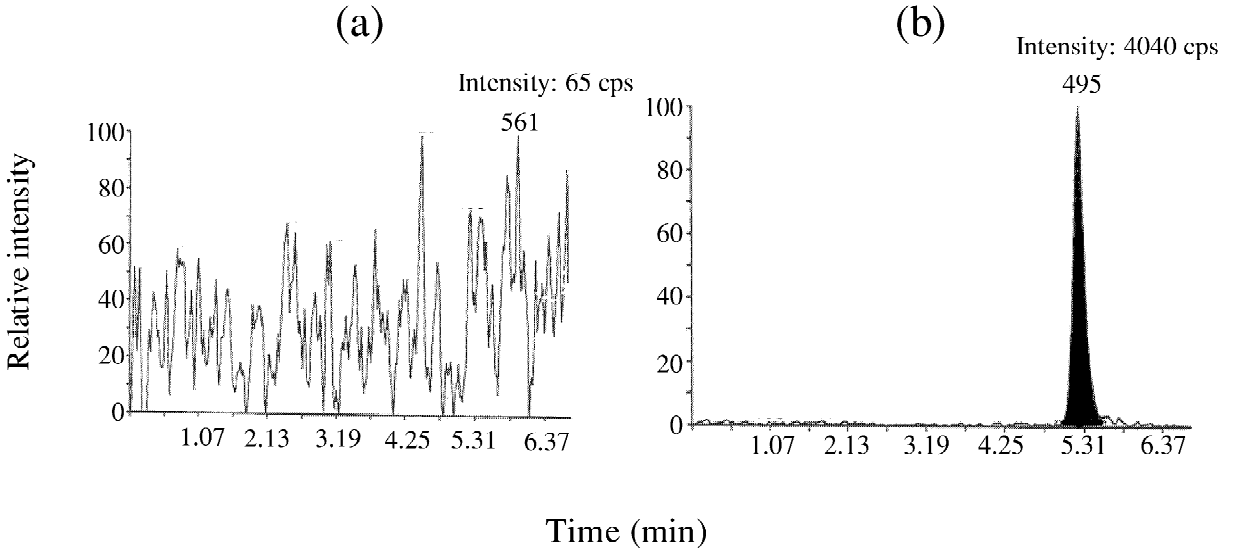


Fig. 3. Ion chromatograms (m/z 328.2 to 310.1 amu) for (a) drug-free plasma and (b) plasma spiked with 13.7 pg/ml of butorphanol (the concentration representing the lower limit of quantitation).

cient of variation (precision) of 17.6%. A representative LLQ ion chromatogram for butorphanol is shown in Fig. 3b.

The number of quality control samples which exhibited deviations of <15% (predicted:nominal) were 8 of 12 at 41.2 pg/ml, and 12 of 12 at 206 and 1031 pg/ml. The accuracy for all three quality controls was within 2% relative standard deviation. The within-run precisions were 3.9, 3.3 and 5.4% at quality control concentrations of 41.2, 206 and 1031 pg/ml, respectively. The between-run precisions were 2.7, 6.5 and 6.8% for concentrations of 41.2, 206 and 1031 pg/ml, respectively. The range of concentration values obtained for all three quality control standards deviated from −5.0 to 15.2% of freshly prepared samples.

As has been reported previously [9], butorphanol was stable in human plasma at room temperature for 24 h and for 3 freeze/thaw cycles.

3.3. Single intranasal dose pharmacokinetic study

The assay was able to quantify butorphanol in the plasma of two healthy subjects for at least 24 h following a single 1 mg intranasal dose. Their concentration versus time profiles are shown in Fig. 4. For Subject 1 and 2, the maximum plasma concentrations of butorphanol were 1474 and 1329 pg/ml, respectively, occurring at 0.25 and 0.5 h post-dose, respectively. The areas under the plasma

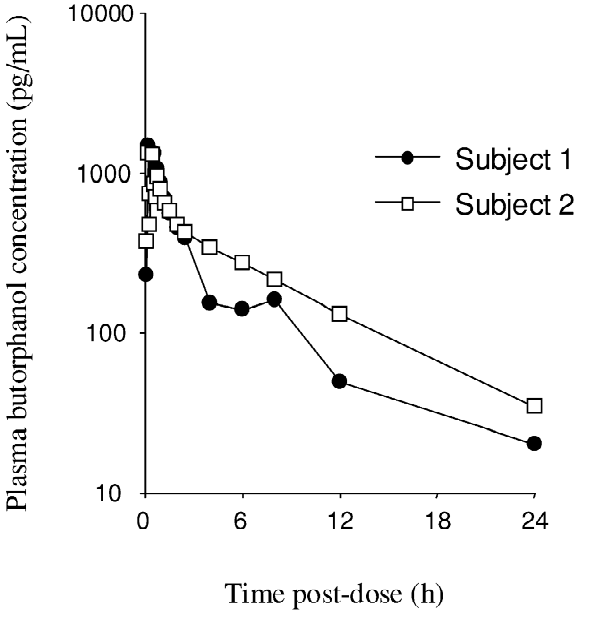


Fig. 4. Plasma concentration–time profile for butorphanol in two healthy subjects following a single 1 mg intranasal dose of butorphanol tartrate.

concentration versus time curves for Subjects 1 and 2 were 6362 and 9207 pg.h/ml, respectively, and their apparent plasma half-lives of butorphanol were 6.0 and 10.0 h, respectively. These pharmacokinetic parameters for intranasal butorphanol were similar to the values obtained by other analytical methods [7,9].

4. Summary

The HPLC–MS/MS assay method described here allowed quantitation of butorphanol in the low pg/ml range and was shown to be able to follow the pharmacokinetics of butorphanol for 24 h following a single 1 mg intranasal dose to two healthy subjects The assay is sensitive, specific, precise, and accurate according to commonly accepted criteria [10]. This assay will therefore, be useful in pharmacokinetic studies using single or multiple doses of butorphanol including those that are designed to examine concentration–response relationships, formulation bioequivalence or potential drug–drug interactions. In addition, this assay is a more convenient alternative to previously reported RIA and GC–MS methods for detecting butorphanol at therapeutic concentrations in human plasma, as it avoids the use of radioactivity or sample derivatization and it possesses the added selectivity and specificity of MS/MS detection.

Acknowledgements

The assistance of Anthony F. Grigor, Ph.D. (Savanet Inc., Savage, MD, USA) and Leonard Lachman, M.D. (Phoenix International Life Sciences, Inc., Neptune, NJ, USA) in assisting with analytical and clinical aspects, respectively, of this study is gratefully acknowledged.

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