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Determination of nalbuphine by high-performance liquid chromatography with ultraviolet detection: application to human and rabbit pharmacokinetic studies

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Abstract

A rapid, sensitive, precise and accurate high-performance liquid chromatographic assay with ultraviolet detection was developed for the determination of nalbuphine in human, rabbit, pig and dog plasma. It is comprised of only a one-step extraction procedure with hexane–isoamyl alcohol at pH 9.25 and reversed-phase chromatography on a μPorasil column. The recoveries of nalbuphine and ethylmorphine (internal standard) were greater than 86%. Calibration graphs were linear over the concentration range 0.75–150 ng/ml with a coefficient of variation, both within-day and between-day, of less than 10% at any level. The limit of quantitation was 0.75 ng/ml of plasma based on a signal-to-noise ratio of 3. Seven other clinically used analgesics were investigated to check for potential interferences and their analytical conditions. The specificity of this assay was checked with a metabolite of nalbuphine (noroxymorphine). Nalbuphine in plasma did not decompose significantly at −20°C for six weeks. Pharmacokinetic application in three surgical patients and four rabbits revealed that nalbuphine followed a linear three-compartment model with two distribution phases. The two distribution and one elimination half-lives and the plasma clearance of nalbuphine were 0.9, 5.8 and 157 min and 370 ml/min in human, and 3.5, 28 and 117 min and 21 166 ml/min in rabbits.

Keywords: Nalbuphine

1. Introduction

Nalbuphine, (−)-17-(cyclobutylmethyl)-4,5α-epoxymorphinan-3, 6α,14-triol, is a relatively new morphine-like drug with partial agonist activity at the κ-opiate receptor and antagonist activity at the μ-opiate receptor [1,2]. As an analgesic agent, it is almost as potent as morphine and has been widely used in the treatment of acute and chronic pain [1–3]. Its main advantages over morphine are a ceiling effect of respiratory depression, low tolerance liability and a lack of significant withdrawal symp-

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toms [4]. It is available as an injection for intramuscular and intravenous administration. The usual recommended doses are 10–20 mg by intravenous or intramuscular injection every 3–4 h [1].

Many analytic methods have been reported in the analysis of nalbuphine in biologic fluids. The gas chromatographic (GC) method reported by Weinstein et al. [5] although sensitive, is much too time-consuming to be used in a pharmacokinetic study involving serial blood samples in a large patient population. Kintz et al. [6] reported a capillary column GC procedure combined with mass spectrometry (MS) and high-performance liquid chromatography (HPLC) combined with diode-array detection. The limits of quantitation were not sensitive and reported to be 2.0 ng/ml for GC–MS and 25 ng/ml for HPLC–diode-array detection. The HPLC with electrochemical detection (ED) has been used widely in the analysis of nalbuphine [7–11]. In these reports, the low quantitation limits of nalbuphine were shown to be of 1 ng/ml [7,11], 0.1 ng/ml [8,9] or 50 pg/ml [10] using 1–3 ml of plasma. Although HPLC with ED was quite sensitive in the analysis of nalbuphine in biological fluids, the stability of ECD still remains the major obstacle in processing HPLC. Ultraviolet (UV) detection was more stable than ED in processing HPLC. Otherwise, the minimal effective concentration of nalbuphine for pain relief was shown to be 20 ng/ml [3]. Therefore, any analytical method which can provide a low quantitation limit of lower than 20 ng/ml may satisfy clinical needs. In the study, we developed a simple, rapid, sensitive, precise and accurate HPLC method (with UV detection) which consists of a one-step extraction procedure with a low quantitation limit of 0.75 ng/ml nalbuphine in human, rabbit, pig and dog plasma. The stability of nalbuphine in frozen human plasma and pilot pharmacokinetic studies in patients and rabbits were also investigated to test the suitability of this method for clinical use.

2. Experimental

2.1. HPLC conditions

The HPLC system consisted of a pump (LC-10AD, Applied Shimadzu, Japan), an automatic sampler (SIL-9A, Shimadzu), a UV detector (SPD-10A, Shimadzu), and an integrator (C-R6A Chromatopac, Shimadzu). A μPorasil column (300 × 3.9 mm, 10 μm particle size, Waters) was used. For the column system, a pre-column (15 × 3.2 mm I.D., 7 μm particle size, Applied Biosystems, San Jose, CA, USA) was also used.

2.2. Chemicals and reagents

Nalbuphine and noroxymorphine (Fig. 1) were kindly supplied by the manufacturer, E.I. du Pont de Nemours, Wilmington, DE, USA. Ethylmorphine was purchased from the Narcotic Bureau, Department of Health, Executive Yuan, Taiwan. All chemicals were of analytical reagent grade. All solvents were of HPLC grade. All aqueous solutions were prepared using Milli-Q water (Milli-RO 60, Millipore, Bedford, MA, USA).

2.3. Standard solutions

Nalbuphine

A stock solution of 240 μg/ml nalbuphine was prepared in water and seven standard solutions of 6.0 μg/ml to 15 ng/ml in water were made by serial dilution. Aliquots of these standard solutions (50 μl

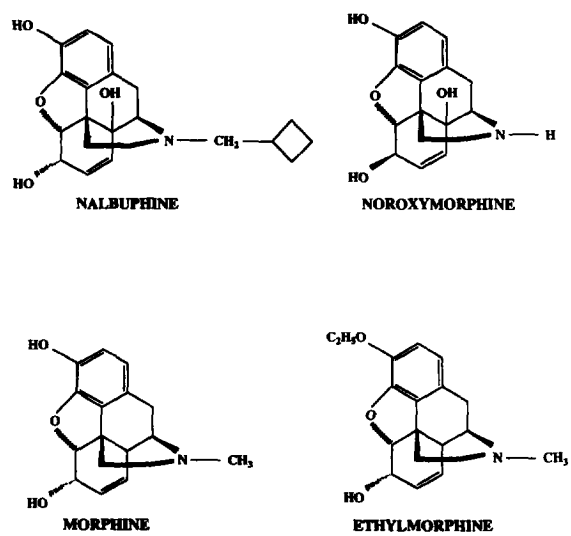

Fig. 1. Structure of nalbuphine, its metabolite (noroxymorphine) and related morphinans.

in humans, pigs or dogs and 25 μl in rabbits) were added to aliquots of blank plasma (0.95 ml in humans, pigs or dogs, and 0.475 ml in rabbits) to give final concentrations of 0.75, 3.75, 7.50, 18.8, 37.5, 75.0 and 300 ng/ml.

Internal standard

A 1.0 mg/ml ethylmorphine stock solution was prepared in water and further diluted to give a working solution of 1 μg/ml in water. A 50-μl aliquot (50 ng) was added to each 1-ml aliquot of human, pig or dog plasma standard or specimen, and 25 μl (25 ng) to each 0.5 ml of rabbit plasma.

2.4. Sample preparation

To 1 ml of human, pig or dog and 0.5 ml of rabbit plasma placed in a 10-ml capacity glass culture tube, fitted with a PTFE-lined screw cap, 50 or 25 μl of internal standard solution and 1 or 0.5 ml of 0.5 *M* sodium carbonate buffer (pH 9.25) were added. The samples were extracted with 3 or 1.5 ml of a mixture of hexane–isoamyl alcohol (9:1, v/v) by mixing for 20 min on a rotary shaker. After centrifugation at 1880 *g* for 15 min, the glass tubes were put into a freezer (−20°C) for 2 h. After the lower layer (plasma) was frozen, the organic layer was poured into another 10-ml glass tube and evaporated to dryness under a stream of filtered dry air. The samples were reconstituted by 125 μl of mobile phase. Aliquots of 100 μl were injected into the HPLC system.

2.5. Chromatography

The assay for nalbuphine was performed using a mobile phase of 5 m*M* sodium acetate buffer (pH 6)–acetonitrile (40:60, v/v) and a UV detector (210 nm). A flow-rate of 1.2 ml/min at 20°C was used and yielded a back-pressure of about 60 bar.

2.6. Calibration graphs

Calibration graphs were obtained by the assay of extracts of blank plasma samples spiked with nalbuphine to cover the concentration range 0.75–300 ng/ml and the internal standard. Quantitation was obtained by the measurement of drug concentrations against the peak-area ratio of nalbuphine/ethylmorphine. The concentrations of unknown samples were determined by using the linear regression line (unweighted) of the concentration of the calibration standard versus peak-area ratios.

2.7. Repeatability, precision and accuracy

The repeatability of the method was estimated by comparing the linear regression slopes, intercepts and correlation coefficients of the standard graphs from human plasma. Precision and accuracy were determined by processing spiked human samples at seven concentrations (0.75–300 ng/ml) with respect to a calibration graph prepared each day. The precision of the method was expressed as the within-day and between-day coefficient of variation (%). The between-day precision was determined by processing spiked human samples at seven concentrations prepared on fifteen separate days. All samples for this purpose were freshly prepared and processed daily (including preparing the standard solution and the extraction procedure). The accuracy of this analytic method was determined by a method demonstrated in previous reports [10–12] and was shown as the mean deviation of all concentrations from theoretical value.

2.8. Selectivity and resolution

To determine the selectivity and resolution of this HPLC system, one of the major metabolites of nalbuphine–noroxymorphine (14-hydroxy-7,8-dihydronormorphine) was detected. Certain drugs which are commonly used clinically during anesthesia and in the post-operative period were also selected to determine the selectivity and resolution of this HPLC system. These included thiopentone, propofol, midazolam, ketamine, succinylcholine, pancuronium, atracurium, diazepam, atropine, glycopyrrolate, neostingmine, xylocaine, apresoline and furosemide.

2.9. Stability and recovery

The stability of nalbuphine in mobile phase and in human plasma were determined. To determine the stability of nalbuphine in mobile phase at room

temperature (20°C), aliquots of seven standard nalbuphine solutions (50 μl) were added to 0.95 ml of mobile phase and were injected into the HPLC system immediately and at 2 h, 4 h and 24 h. To determine the stability of nalbuphine in human plasma, aliquots of seven standard nalbuphine solutions (50 μl) were added to a batch of blank human plasma (0.95 ml), and then incubated under 37°C water bathing or stored under −20°C freezing. The nalbuphine concentration was determined at 2 and 4 h after warm water bathing, and three and six weeks after freezing. The nalbuphine calibration graph was constructed for each experiment. The results of the stability studies were analyzed using a 90% confidence-interval approach [13].

The extraction recovery of nalbuphine and the internal standard in human plasma was determined at all levels of the calibration graph by comparing the peak areas obtained by the direct injection of standard aqueous solutions to those obtained after the whole extraction procedure.

2.10. Pharmacokinetic studies

Humans

This analytical method has been used in a preliminary pharmacokinetic study in humans. After institutional approval and patient agreement, three surgical patients undergoing thoracotomy for benign tumor excision received intravenous nalbuphine (0.16 mg/kg) to relieve postoperative pain. In the post-anesthetic room, 15 ml of blood were collected from a forearm vein into a heparinized tube at zero time and 2 ml at 2, 5, 10, 15, 30, 60, 90, 120, 240 and 360 min after the intravenous administration of nalbuphine hydrochloride.

Animals

Following the guidelines of the American Association for the Accreditation of Laboratory Animal Care, four six-month-old male New Zealand white rabbits weighing between 3.0 and 3.3 kg were used. After placing the animals in a restraining box, their ears were shaved with a razor blade. Following the successful cannulation of the auricular artery and vein in the opposite ear, 7 ml of blood were collected from the artery at zero time and 1 ml at 2, 5, 10, 15, 20, 30, 45, 100, 120, 180, 240 and 360 min after the intravenous injection of nalbuphine hydrochloride (10 mg/kg).

Both human and rabbit plasma were collected by centrifugation and immediately frozen to −20°C until assay. Plasma concentrations were fitted to a three-compartment open model using the computer program PCNONLIN, version 3.0 [14]. Akaike information criteria (AIC), weighted residual sum of squares, and residual plots were used to judge the goodness-of-fit of the model to data. Initial parameter estimations were required for non-linear regression and were obtained using the linear regression computer program CSTRIP [15]. Pharmacokinetic parameters such as half-lives, clearance, apparent central compartment volume of distribution and area under the plasma concentration time graph were calculated for patients and rabbits by standard formulae [16].

3. Results and discussion

3.1. Chromatography

As the chromatograms of extracts from humans, rabbits, pigs and dogs are similar, only the typical chromatograms of extracts from human patients are shown (Fig. 2) These are extracts of drug-free

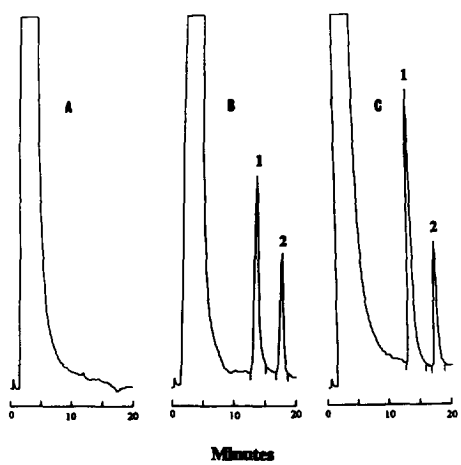

Fig. 2. Chromatograms of extracts from (A) blank plasma, (B) plasma spiked with nalbuphine (90 ng/ml) and (C) sample from a patient (nalbuphine 120 ng/ml). Peaks: 1, nalbuphine; 2, internal standard (ethylmorphine 50 ng/ml).

plasma, spiked samples with nalbuphine and the internal standard, ethylmorphine. No interfering peaks were detected in the blank plasma nor in samples from the patients or animals.

3.2. Low quantitation limit, column retention time, capacity factors and column selectivity of various narcotics

The low quantitation limit, retention times, capacity factors and column selectivity of nalbuphine and the other analgesics listed in Table 1, which are widely used clinically, were investigated to check for their potential interference and their respective analytical conditions.

In different column tests, the μPorasil column exhibited a great selectivity. Using the mobile phase buffer–acetonitrile (40:60), nalbuphine and the internal standard had retention times of 13.94 and 17.82 min, respectively. The low quantitation limits of nalbuphine, defined as a signal-to-noise ratio greater than 3, was 0.75 ng/ml of plasma, respectively. Also, nalbuphine could be determined by elution with various ratios of buffer (5 m*M* sodium acetate, under different pH values)–acetonitrile and the result is shown in Fig. 3. Under these elution conditions, the retention times of nalbuphine and internal standard–ethylmorphine were kept nearly constant.

Table 1
Retention times, capacity factors, column selectivity and low detection limits of various drugs on a silica gel column

Drug	t_R	k'	A	Limit of quantitation (ng/ml)
Nalbuphine	13.94	5.97	1.00	0.75
Ethylmorphine	17.82	7.92	1.28	1.00
Noroxymorphine	22.06	10.03	1.58	0.75
Buprenorphine	7.66	2.83	0.55	1.00
Morphine	20.55	9.28	1.47	1.00
Fentanyl	9.67	3.84	0.69	4.70
Meperidine	15.36	6.68	1.10	6.30
Codeine	19.52	8.76	1.40	0.80
Tramadol	16.23	7.12	1.16	6.00
Naloxone	6.21	2.10	0.44	1.00

Conditions: mobile phase, 5 m*M* sodium acetate buffer (pH 6)–acetonitrile (40:60); flow-rate, 1.2 ml/min; Shimaze SPD-10A UV detector (210 nm); temperature, 20°C. t_R, retention time (min); k', capacity factory; A, column selectivity compared with nalbuphine.

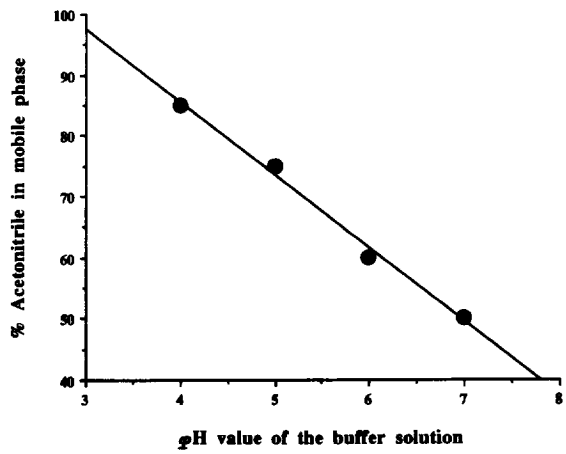

Fig. 3. Relationship between the pH value of the buffer solution (5 m*M* sodium acetate) and the ratio of the percentage of acetonitrile–buffer. Under these elution conditions, the column retention times of nalbuphine and internal standard–ethylmorphine were kept nearly constant.

3.3. Selectivity and resolution

The selectivity and resolution of this HPLC system was challenged by co-administration of nalbuphine with its metabolite–noroxymorphine. The result showed that it is quite easy to differentiate nalbuphine from its metabolite, noroxymorphine, by different capacity factors of 5.97 and 10.03 and selectivity factors of 1 and 1.58, respectively. Certain drugs which are commonly used clinically during anesthesia and in the post-operative period were selected to check for their potential interference in the assay of nalbuphine. These included thiopentone, propofol, midazolam, ketamine, succinylcholine, pancuronium, atracurium, diazepam, atropine, glycopyrrolate, neostigmine, xylocaine, apresoline and furosemide. After injection directly into the HPLC system, no interfering peaks were found during the analysis.

3.4. Repeatability, precision and accuracy

Over a period of 92 days, the calibration graphs ($n = 18$) were linear in the concentration range 0.75–300 ng/ml with correlation coefficients of 0.998±0.001 (mean±S.D.) and with a minimum intercept of 0.28±0.11 (mean±S.D.). The slopes averaged 180±15 ng/ml with a coefficient of vari-

ation of 8.3%. Precision and accuracy studies in plasma showed an acceptable coefficient of variation (<10%) and high accuracy for both within-day (n = 6) and between-day (n = 15) studies, as shown in Table 2.

3.5. Stability and recovery

The stability of nalbuphine in mobile phase for 24 h, in plasma under warm water bath at 37°C for 4 h and in plasma after freezing at −20°C for six weeks were determined and demonstrated in Tables 3–5. The results indicate that no significant degradation occurred under different treatments. Dube et al. [11] indicated that nalbuphine was stable for seven weeks of freezing at −20°C. Lo et al. [9] also predicted that nalbuphine was stable in frozen plasma for at least eighteen weeks. The absolute recoveries of nalbuphine and the internal standard were greater than 86% and independent of the nalbuphine concentration (Table 6). Compared with other extracted organic solvents or mixtures such as dichloromethane, benzene, diethyl ether, chloroform or hexane–isopropanol, the mixture hexane–isoamyl alcohol gave a greater recovery and chromatograms with less background noise for both human and animal samples. The extraction procedure, involving only one step, is fairly rapid and the freezing method also simplified the procedure of solvent transfer. This allows the analysis of at least eighty samples per day using the automated HPLC system.

Table 3
Stability of nalbuphine in mobile phase

Initial concentration (ng/ml)	Concentration found in samples (ng/ml)		
	2 h	4 h	24 h
0.76±0.05	0.79±0.08	0.81±0.08	0.78±0.06
3.89±0.29	4.03±0.31	4.02±0.37	4.02±0.35
7.30±0.61	7.12±0.8	7.21±0.38	7.31±0.62
18.7±1.5	18.2±1.3	18.3±1.2	18.2±1.4
38.1±2.1	38.4±1.7	38.9±1.7	38.6±1.8
75±3.8	77.1±3.4	77.9±3.6	75.1±4.1
301±14	307±12	303±12	302±15

Mean±S.D., n = 6 each; there is no statistical difference between the initial concentrations and samples at each test time using a 90% confidence interval approach.

3.6. Pharmacokinetic studies

The HPLC method has been used intensively in our laboratories. The observed and fitted plasma

Table 2
Precision and accuracy of nalbuphine determined by the HPLC method

Known concentration (ng/ml)	Concentration found (mean±S.D.) (ng/ml)	Coefficient of variation (%)	Accuracy (% mean deviation)
Within-day (n = 6)			
0.75	0.80±0.06	7.5	6.7
3.75	4.01±0.25	6.2	6.9
7.50	7.20±0.40	4.0	−4.0
18.8	18.0±1.2	6.4	−4.3
37.5	39.2±1.4	3.6	4.5
75.0	77.1±3.2	4.2	2.8
300	306±8	3	2
Between day (n=15)			
0.75	0.82±0.08	9.8	9.3
3.75	402±0.38	9.4	7.2
7.50	7.30±0.60	8.2	−3.7
18.8	18.1±1.6	8.4	−3.8
37.5	38.5±2.1	5.5	2.7
75.0	79.1±4.1	5.2	5.5
300	308±10	3	3

Table 4
Stability of nalbuphine in plasma under 37°C water bath

Concentrations in standard curve (ng/ml)	Concentrations found in samples (ng/ml)	
	2 h	4 h
0.78±0.07	0.80±0.06	0.81±0.07
3.86±0.26	4.01±0.78	4.02±0.37
7.60±0.52	7.10±0.51	7.21±0.42
18.6±1.5	18.1±1.4	18.2±1.1
37.9±1.6	38.4±1.6	39.1±1.8
78.1±3.4	77.1±3.2	78.1±3.4
306±11	306±10	304±12

Mean±S.D., $n = 6$ each; there is no statistical difference between standard curves and samples at each test time using a 90% confidence interval approach.

Table 5
Stability of nalbuphine in frozen plasma (−20°C)

Nominal concentration (ng/ml)	21 days (ng/ml)		42 days (ng/ml)	
	Standard curve	Sample	Standard curve	Sample
0.75	0.78±0.05	0.80±0.06	0.81±0.08	0.81±0.07
3.75	4.02±0.30	4.03±0.36	4.06±0.38	4.02±0.38
7.50	7.14±0.51	7.31±0.62	7.22±0.41	7.24±0.72
18.8	18.4±1.2	18.2±1.4	18.3±1.2	18.5±1.2
37.5	38.1±1.7	38.6±1.9	39.2±1.7	37.8±2.1
75	77.3±3.1	77.4±4.1	78.1±3.6	78.2±4.3
300	301±11	308±16	305±12	300±14

Mean±S.D., $n = 6$ each; there is no statistical difference between standard curves and samples at each test time using a 90% confidence interval approach.

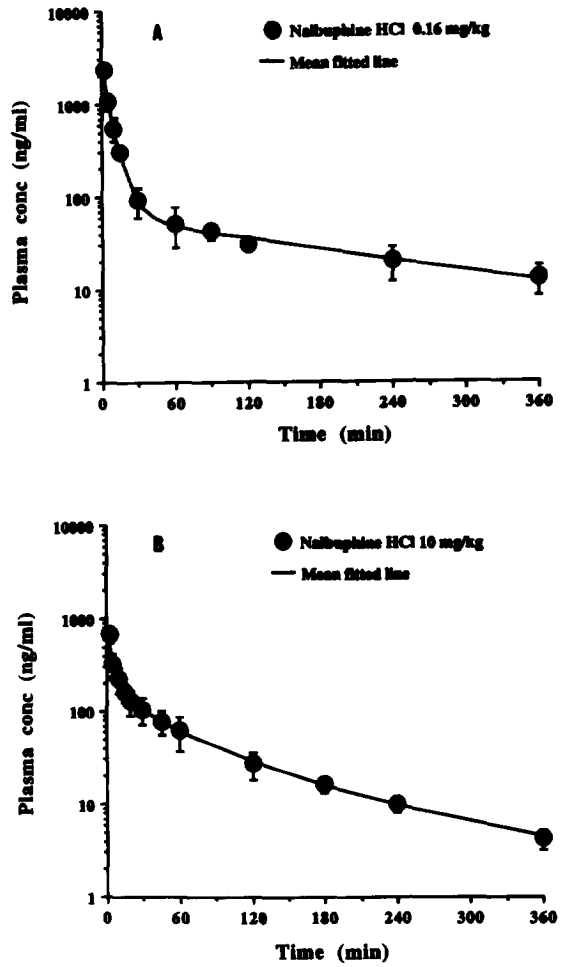

Fig. 4. Plasma concentration–time profiles of nalbuphine in three surgical patients (A) and four rabbits (B) receiving intravenous nalbuphine HCl.

Table 6
Absolute recoveries of nalbuphine and ethylmorphine (internal standard) from spiked plasma samples ($n = 6$)

Drug	Concentration (ng/ml)	Recovery (mean±S.D.) (%)	Coefficient of variation (%)
Nalbuphine	0.75	87.1±4.6	5.3
	3.75	90.1±4.3	4.8
	7.50	89.5±5.2	5.8
	18.8	91.2±3.8	4.2
	37.5	89.4±4.6	5.1
	75.0	89.2±3.5	3.9
	300	92±5	5.0
Ethylmorphine	50.0	86.2±5.1	5.9

Table 7
Pharmacokinetic parameters of nalbuphine in three surgical patients and four rabbits after intravenous nalbuphine injection

Parameter[a]	Unit	Human	Rabbit
Dose	mg/kg	0.17	10
A	ng/ml	6462±2545	554±328
B	ng/ml	1794±751	147±57
C	ng/ml	58.7±3.5	48.7±24.3
α	1/min	0.948±0.405	0.252±0.166
β	1/min	0.126±0.031	0.028±0.013
γ	1/min	0.0044±0.0004	0.0064±0.0022
$T_{1/2(\alpha)}$	min	0.86±0.49	3.45±1.42
$T_{1/2(\beta)}$	min	5.8±1.7	27.8±10.4
$T_{1/2(\gamma)}$	min	157±12	117±46
$AUC_{0-\infty}$	ng min/ml	29754±10003	15164±3498
Cl_t	ml/min	370±154	21166±629
Body weight	kg	63.7±5.1	3.1±0.2

[a]Equation: plasma concentration $(C_p) = Ae^{-\alpha\tau} + Be^{-\beta\tau} + Ce^{-\gamma\tau}$; A, B, C, intercepts; α, β, γ are the first-order rate constants for the central, tissue, and deep tissue compartment; $T_{1/2}$, half-life of the first-order rate constant; $AUC_{0-\infty}$, area under the time–concentration graph to time infinity; Cl_t, total plasma clearance.

concentration–time curves for the intravenous injection of nalbuphine into three human patients after thoracotomy and four rabbits are shown in Fig. 4. The data were successfully fitted to a linear three-compartment model with two distribution phases and one elimination phase. The plasma level of intravenous nalbuphine declined very rapidly in patients and rabbits, as expected for this lipophilic drug. Slow elimination phases were found with elimination half-lives of 157 and 117 min in patients and rabbits, respectively (Table 7).

4. Conclusion

This method is sufficiently simple, rapid, sensitive, specific and accurate for the determination of nalbuphine in large amounts of human and animal biological fluids. It has been extensively used in our laboratories for pharmacokinetic studies of the drug in human patients and animals.

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References

[1] W.K. Schmidt, S.W. Tam, G.S. Schotzberger, D.H.J. Smith, R. Clark and V.G. Vernier, Drug Alcohol. Depend., 14 (1985) 339.
[2] C.G. Pick, D. Paul and G.W. Pasternak, J. Pharmacol. Exp. Ther., 262 (1992) 1044.
[3] G.C. Pugh and G.B. Drummond, Br. J. Anaesth., 59(1987) 1356.
[4] K.L. Preston and D.R. Jasinski, Drug Alcohol. Depend., 28 (1991) 49.
[5] S.H. Weinstein, M. Alteras and J. Gaylord, J. Pharm. Sci., 67 (1978) 547.
[6] P. Kintz, A. Tracqui and P. Mangin, J. Chromatogr., 579 (1992) 172.
[7] C.L. Lake, C.A. DiFazio, E.N. Duckworth, J.C. Moscicki, J.S. Engle and C.G. Durbin, J. Chromatogr., 233 (1982) 410.
[8] M. Keegan and B. Kay, J. Chromatogr., 311 (1984) 223.
[9] M.W. Lo, G.P. Juergens and C.C. Whitney, Res. Commun. Chem. Pathol. Pharmacol., 43 (1984) 159.
[10] N. Wetzelsberger, P.W. Lücker and W. Erking, Arzneim.-Forsch./Drug Res., 38(II), Nr.12 (1988) 1768.
[11] L. Dube, N. Beaudoin, M. Lalande and I.J. McGilveray, J. Chromatogr., 427 (1988) 113.
[12] S.T. Ho, J.J. Wang, W. Ho and O.Y.P. Hu, J. Chromatogr., 570 (1991) 339.
[13] U. Timm, M. Wall and D. Dell, J. Pharm. Sci., 74 (1985) 972.
[14] C.M. Metzler and D.L. Weiner, PCNONLIN User's Guide, Statistical Consultants, Lexington, KY, 1989.
[15] A.J. Sedman and J G. Wagner, J. Pharm. Sci., 65 (1976) 1006.