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Journal of Chromatography B, 714 (1998) 237–245

JOURNAL OF
CHROMATOGRAPHY B

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

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Received 30 December 1997; received in revised form 29 April 1998; accepted 1 May 1998

Abstract

A rapid, sensitive, precise and accurate high-performance liquid chromatographic assay with coulometric electrochemical detection was developed for the determination of morphine in human, rabbit, pig and dog plasma. It includes a one-step extraction procedure with hexane–isoamyl alcohol (1:1, v/v) at pH 8.9 (adjusted with phosphoric acid) and reversed-phase liquid chromatography on a μPorasil column. The mobile phase was composed of 5 m*M* sodium acetate buffer (pH 3.75)–acetonitrile (25:75, v/v). A flow-rate of 1.2 ml/min at 20°C was used. The working potentials for the electrochemical detector were +0.20 V for detector cell 1, +0.55 V for detector cell 2 and +0.75 V for the guard cell. The limit of detection of morphine was 100 pg/ml of plasma. Repeatability, precision and accuracy were also determined concomitantly. The calibration graphs were linear in the concentration range 0.25–250 ng/ml with correlation coefficients of 0.998±0.01 and with a minimum intercept of 0.05±0.08. The precision in plasma was acceptable, with coefficients of variation less than 11%. The absolute recoveries of morphine and nalbuphine (internal standard) were between 86 and 89% and independent of morphine concentration. Pharmacokinetics after oral morphine [MST Continus™ (morphine sulphate tablets) 30 mg, Bard Pharmaceutical, Cambridge, UK] in humans revealed a one-compartment first-order absorption model with one absorption phase and one elimination phase. The absorption and elimination half-lives were 2.46 and 1.80 h, respectively. Pharmacokinetics after intravenous morphine (3 mg/kg) in rabbits showed a linear two-compartment open model with one distribution phase and one elimination phase. The distribution and elimination half-lives were 0.5 and 33.8 h, respectively.
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Keywords: Morphine

1. Introduction

Morphine has a major effect on the central and peripheral nervous system by acting on the opioid receptors. The effects include analgesia, sedation, hypotension, drowsiness, respiratory depression, nausea, vomiting, and alterations of the endocrine and autonomic nervous system [1]; potentially fatal

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PII: S0378-4347(98)00230-8

effects are respiratory depression and cardiovascular instability [1]. Due to a narrow 'therapeutic window' of the drug, monitoring of morphine concentration in plasma may be necessary [2–9].

Radioimmunoassay (RIA) [2–6] is the most widely used method for quantitation of morphine in clinical environments. RIA is sensitive and can measure morphine concentrations in ng/ml range; however, antibody cross-reactivity with glucuronide metabolites and other opioids limit the selectivity of the method [2–6]. The combined technique of high-performance liquid chromatography (HPLC) followed by RIA has been reported but it is a very time-consuming procedure, and inconvenient for routine application [2–4]. Gas chromatographic methods (GC), particularly coupled to mass spectrometric detection, have proved suitable for morphine assay being sensitive and selective, but require prior derivatisation, expensive and delicate equipment [3,5,10,11]. Several HPLC methods have also been reported to determine the morphine concentration in biological fluids with the use of ultra-violet (UV) radiation absorption or fluorometric detection [12,13]; however, these methods are not sufficiently sensitive for determining therapeutic concentrations. Meanwhile, several reports have proposed the use of HPLC with electrochemical detection (ED). These methods are sufficiently selective and sensitive; limit of quantitation was 1 ng/ml with amperometric instruments [14,15] and 200–240 pg/ml with coulometric methods [16,17]. Recently, a quite low level of quantitation (50 pg/ml) in human plasma with the use of HPLC–ED was also reported [18]. However, no details of the detector were noted and a multiple extraction procedure was needed for morphine determination. In the current study, we have achieved the analytical sensitivity of 100 pg/ml with a one-step extraction. Pilot pharmacokinetic studies in normal healthy volunteers and rabbits were also carried out to test the suitability of this method for clinical use.

2. Experimental

2.1. HPLC apparatus

The HPLC system consisted of a Model LC-10AD pump (Shimadzu, Japan), a SIL-9A automatic sampler (Shimadzu), a 5200A Coulochem electrochemical detector (ESA, Bedford, MA, USA), equipped with a Model 5010 analytical cell and a Model 5020 guard cell, and a C-R6A integrator (Shimadzu). A 300×3.9 mm I.D. μPorasil column (10 μm particle size, Waters, Milford, MA, USA) protected by a 15×3.2 mm I.D. precolumn (7 μm particle size, silica packed, Applied Biosystems, San Jose, CA, USA) was used.

2.2. Chemicals and reagents

Morphine and its metabolites [morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G)] (Fig. 1) were kindly supplied by the National Narcotics Bureau, Department of Health, Taiwan. Nalbuphine (internal standard) was kindly supplied by the manufacturer (E.I. du Pont de Nemours, Wilmington, DE, USA). All chemicals were of analytical reagent grade. All solvents were of HPLC grade. All aqueous solutions were prepared by using Milli-Q water (Milli-RO 60, Millipore, Bedford, MA, USA).

2.3. Standard solutions

2.3.1. Morphine

A stock solution of morphine was prepared in water with a concentration of 25 μg/ml and six standard solutions from 5 ng/ml to 5 μg/ml in water

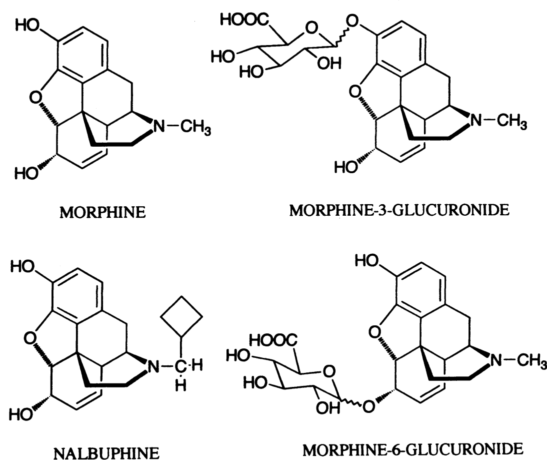

Fig. 1. Structure of morphine, its metabolites (morphine-3-glucuronide and morphine-6-glucuronide) and nalbuphine (internal standard).

were made by serial dilution. Aliquots of these standard solutions (5% of the final volume) were added to blank plasma to give final concentrations of 0.25, 2.50, 7.50, 25.0, 75.0 and 250 ng/ml.

2.3.2. Internal standard

A 1.0 mg/ml nalbuphine 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 every 1 ml aliquot of human, pig or dog plasma standard or specimen, and 25 μl (25 ng) to every 0.5 ml of rabbit plasma.

2.4. Sample preparation

All glassware used was silanized to prevent the adsorption of opioids onto the walls. This was accomplished by immersing the glassware in a 5% solution of dichlordimethysilane (Merck, Schuchardt, Hohenbrunn, Germany) in chloroform overnight and then putting the glassware into an oven (100°C) for 3–4 h. The glass tubes used for extraction of morphine from plasma were fitted with a PTFE-lined screw cap. For humans, pigs and dogs, 1 ml of their plasma was mixed with 50 μl of internal standard solution and 1 ml of 0.5 *M* sodium carbonate buffer (pH 8.9, adjusted with 5 *M* phosphoric acid). For rabbits, half of the above solutions were used. The samples were extracted with 4 ml or 2 ml (rabbits) of a mixture of hexane–isoamyl alcohol (1:1, 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 with a refrigerated evaporator (Models VLP 120, SC 110 and RVT 400, Savant, NY. USA). The samples were reconstituted with 125 μl of mobile phase. Aliquots of 100 μl were injected into the HPLC system.

2.5. Chromatography

The assay for morphine was performed using a mobile phase composed of 5 m*M* sodium acetate buffer (pH 3.75)–acetonitrile (25:75, v/v). The mobile phase was saturated with silica powder (Nucleosil, Macherey Nagel, Germany) before use. A flow-rate of 1.2 ml/min at 20°C was used and yielded a backpressure of about 60 bar. The working potentials for the electrochemical detector were +0.20 V for detector cell 1, +0.55 V for detector cell 2 and +0.75 V for the guard cell.

2.6. Calibration

Calibration curves were prepared with blank plasma samples spiked with morphine to cover the concentration range from 0.25 to 250 ng/ml and with the internal standard at the fixed concentration of 50 ng/ml. Calibration graphs were obtained by plotting drug concentrations against the peak-area ratio of morphine/nalbuphine. The concentrations of unknown samples (morphine) were determined by using the linear regression line (unweighted) of the calibration standard.

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 calibration curves from human plasma. Precision and accuracy were determined on spiked human samples at six concentrations (0.25–250 ng/ml) with respect to a calibration graph prepared every day. The precision of the method was expressed as the within-day and day-to-day coefficient of variation (%). The day-to-day precision was determined by processing spiked human samples at six concentrations prepared on 82 separate days. All samples for this purpose were freshly prepared and processed daily, including preparing the standard solution from the same stock solution (25 μg/ml). The accuracy of this analytical method was determined by a method demonstrated in our previous reports [19,20]. Briefly, the accuracy was shown as percent of mean deviation from actual concentration [(concentration found−known concentration)×100/known concentration].

2.8. Selectivity

To determine the selectivity of this HPLC method, two major metabolites of morphine (M3G, M6G) were studied, as well as drugs commonly used during anesthesia and in the postoperative period. These drugs included thiopentone, propofol,

midazolam, ketamine, succinylcholine, pancuronium, atracurium, diazepam, atropine, glycopyrrolate, neostigmine, xylocaine, propranolol and furosemide. Naloxone and analgesics, such as buprenorphine, codeine, fentanyl, pethidine and tramadol were also investigated. A 1-μg amount of the above drugs was added into 1-ml plasma. After extraction, these drugs were injected into the HPLC system to check their potential interference with the assay.

2.9. Recovery

The extraction recovery of morphine 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

2.10.1. Humans

After institutional approval and informed consent, eight healthy young (aged 24.0±2.5) Chinese volunteers enrolled the study. After about 10 h fasting, administration of single (oral) 30 mg of morphine controlled release tablet [MST Continus (morphine sulphate tablets)], along with 200 ml of water took place between 7:30 and 8:00 in the morning. Apart from water, no food and drink was permitted for 4 h after administration. Before taking any medicine, 7 ml of blood was collected from a vein into a heparinized tube, and then, 2 ml was drawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h after drug intake.

2.10.2. Animals

Following the guidelines of the American Association for the Accreditation of Laboratory Animal Care, three 5-month-old male New Zealand white rabbits weighing between 2.6 and 3.1 kg were used. After placing the animal in a restraining box, cannulations of the auricular artery and vein in opposite ears were done. A 5-ml volume of blood was obtained from the artery at time zero and 1 ml at 5, 10, 15, 30 min and at 1, 2, 4, 8, 24 h after intravenous administration of morphine (3 mg/kg). The blood was collected into heparinized tubes (50 μl heparin, 1000 I.U./ml).

Both human and rabbit plasma were obtained by centrifugation and immediately frozen at −20°C until assay. The plasma concentration–time profiles of morphine in healthy volunteers and rabbits were fitted by using the computer program PCNONLIN (version 3.0, Statistical consultants) [21]. 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. A C-strip computer program was used to obtained the initial parameter estimations, which were required for nonlinear regression analysis by the computer program PCNONLIN [22]. Pharmacokinetic parameters such as maximum plasma concentration (C_{max}), time after administration at which C_{max} occurred (t_{max}), half-lives ($t_{1/2}$), clearance (CL), area under the plasma concentration (AUC) and mean residence time (MRT) were calculated by standard formulae [23].

3. Results

3.1. Chromatography

As the chromatograms from humans, rabbits, pigs and dogs were similar, only typical chromatograms from human plasma are shown in Fig. 2. No interfering peaks were detected in the blank plasma or in samples from the human subjects or animals.

3.2. Limit of detection, column retention time, capacity factors and resolution

The limit of detection, retention times, capacity factors, resolution of morphine and other analgesics, which are widely used, were investigated to check for their potential interference (Table 1).

In different column tests, the μPorasil column exhibited a great selectivity. Using the mobile phase buffer–acetonitrile (25:75, v/v), morphine and the internal standard had retention times of 10.45 and 8.21 min, respectively. The limit of detection of morphine, defined at a signal-to-noise ratio greater than 3, was 100 pg/ml of plasma.

Among the tested drugs M3G and M6G were not detectable with ED (Table 1). The others demonstrated no interfering peaks after the same extraction

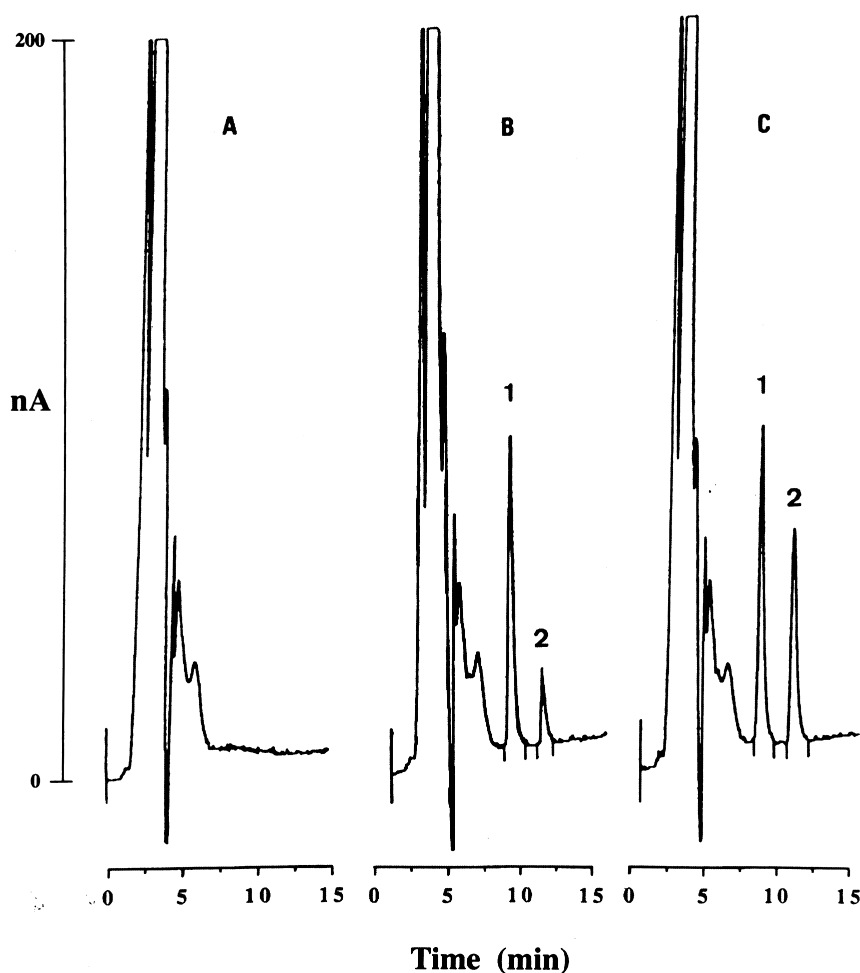

Fig. 2. Chromatograms of extracts from (A) blank plasma, (B) plasma spiked with morphine (7.5 ng/ml) and (C) sample from a healthy volunteer (morphine 30 ng/ml). Peaks: 1=internal standard (nalbuphine 50 ng/ml); 2=morphine. For analytical conditions, see text.

procedures. These included thiopentone, propofol, midazolam, ketamine, succinylcholine, pancuronium, atracurium, diazepam, atropine, glycopyrrolate, neostigmine, xylocaine, propranolol and furosemide.

3.3. Repeatability, precision and accuracy

Over a period of 82 days, the calibration graphs ($n=20$) were linear in the concentration range 0.25–250 ng/ml with correlation coefficients of 0.998±0.001 (mean±S.D.) and with a minimum intercept of 0.05±0.08 (peak area ratio; mean±S.D.) The slopes averaged 1.29±1.21 with a coefficient of variation of 9.1%. Precision studies in plasma proved an acceptable coefficient of variation (<11%). A high accuracy in both within-day ($n=6$) and day-to-day ($n=18$) studies (≤12%) was also demonstrated (Table 2).

3.4. Recovery

Compared to other organic solvents or mixtures tested for extraction (dichloromethane, benzene, diethyl ether, chloroform or hexane–isopropanol), the mixture hexane–isoamyl alcohol (1:1, v/v) gave the best recovery and chromatograms with less background noise for both human and animal samples. The absolute recoveries of morphine and the

Table 1
Retention times, capacity factors, resolution and limit of detection of various drugs

Drug	t_R (min)	k	R (ng/ml)	Limit of detection
Morphine	10.45	5.04	[a]	0.10
Nalbuphine	8.21	3.74	2.24	0.10
Buprenorphine	6.61	2.82	5.15	0.20
Naloxone	8.82	4.10	1.64	0.05
Codeine	8.24	3.76	2.22	0.14
Morphine-3G	–	–	–	–
Morphine-6G	–	–	–	–
Fentanyl	–	–	–	–
Tramadol	–	–	–	–
Meperidine	–	–	–	–

Conditions: see text; t_R=retention time of peak; k=capacity factor=$(t_R-t_0)/t_0$; t_0=retention time of unretained peak; R=resolution=$2(t_2-t_1)/w_2+w_1$.

[a] Morphine is the standard drug for detecting the resolution; t_1=retention time of drug detected; t_2=retention time of morphine; w_1=peak width of drug detected; w_2=peak width of morphine; –=not detectable.

Table 2
Precision and accuracy of morphine HPLC determination

Known concentration (ng/ml)	Concentration found (mean±S.D.) (ng/ml)	Coefficient of variation (%)	Accuracy (% mean deviation)
Within-day (n=6)			
0.25	0.27±0.02	7.4	8
2.50	2.70±0.26	9.6	8
7.50	7.42±0.58	7.8	−1.1
25.0	26.1±2.1	8.0	4.4
75.0	73.1±4.8	6.6	−2.5
250	246±12	4.9	−1.6
Day-to-day (n=18)			
0.25	0.28±0.03	10.7	12
2.50	2.75±0.27	9.8	10
7.50	7.40±0.61	8.2	−1.3
25.0	26.8±1.5	5.6	7.2
75.0	78.2±4.1	5.2	4.3
250	245±14	5.7	−2

Table 3
Absolute recoveries of morphine and nalbuphine (internal standard) from spiked plasma samples (n=8)

Drug	Concentration (ng/ml)	Recovery (%) (mean±S.D.)	Coefficient of variation (%)
Morphine	0.25	86.2±6.1	7.1
	2.50	87.8±6.5	7.4
	7.50	89.2±5.2	5.8
	25.0	86.8±4.6	5.3
	75.0	88.1±3.8	4.3
	250	89.6±5.1	5.7
Nalbuphine	50	90.2±4.1	4.5

internal standard were between 86 and 89%, independently of morphine concentration (Table 3). The one-step extraction procedure is fairly rapid and the freezing method also simplifies the procedure of solvent transfer. This allows the analysis of 80 samples at least per day using an autosampler.

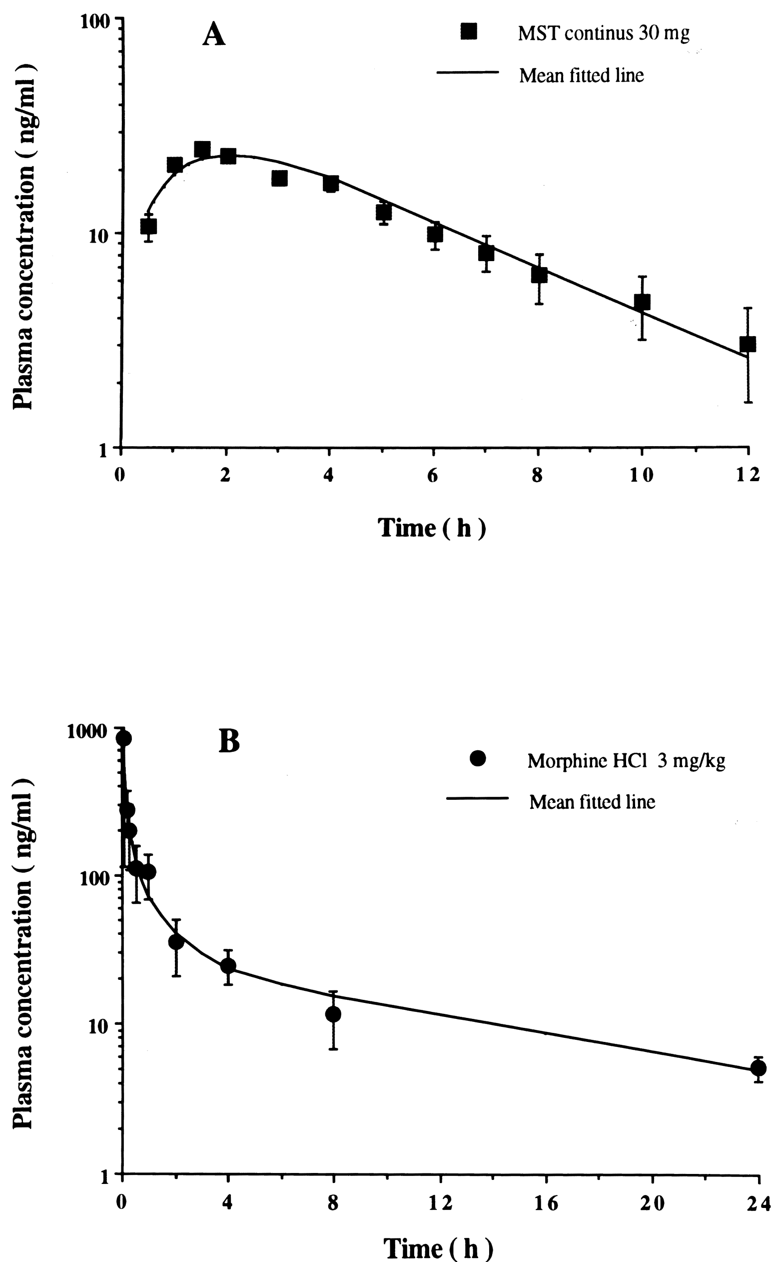

Fig. 3. Plasma concentration–time profiles of morphine in eight healthy volunteers (A) and three rabbits (B) receiving oral morphine (MST continus) or intravenous morphine HCl, respectively.

Table 4
Pharmacokinetic parameters in eight human subjects after single oral administration of controlled release morphine tablet (30 mg)

Subject	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0-\infty}$ (h ng/ml)	$t_{1/2k_a}$ (h)	$t_{1/2k_e}$ (h)	MRT (h)
1	24.8	2.73	173.4	1.89	1.89	5.40
2	18.4	2.01	96.0	1.06	1.89	4.08
3	18.5	1.06	108.3	1.25	3.83	4.26
4	25.1	2.14	177.1	2.86	0.87	5.34
5	25.1	2.16	159.7	2.35	1.01	4.84
6	20.3	1.24	122.6	1.32	3.79	4.44
7	33.8	1.31	203.7	3.01	0.39	4.86
8	18.7	2.45	198.4	5.92	0.70	9.16
Mean	23.1	1.89	154.9	2.46	1.80	5.30
S.D.	5.3	0.61	41.1	1.58	1.35	1.63

C_{max} = maximum plasma morphine concentration; t_{max} = time after administration at which C_{max} occurred; $AUC_{0-\infty}$ = area under the time–concentration graph from time zero to time infinity; $t_{1/2k_a}$ = half-life of absorption rate constant; $t_{1/2k_e}$ = half-life of elimination rate constant; MRT = mean residence time.

3.5. Pharmacokinetic studies

The HPLC method has been used extensively in our laboratories in pharmacokinetic studies. The observed plasma concentration–time curves for morphine in eight normal healthy volunteers and three rabbits are shown in Fig. 3. The data in human were successfully fitted to a one-compartment first-order absorption model with one absorption phase and one elimination phase (Table 4). In the current study, controlled release morphine tablets were used. In this controlled release dosage form, the absorption rate constant (k_a) is always smaller than the elimination rate constant (k_e) [23]; therefore, the terminal phase of an oral absorption curve is usually represented by the absorption rate constant, and the elimination rate constant is represented by the steeper slope [24]. In the current study, the half-lives of absorption and elimination were 2.46 and 1.80 h, respectively (Table 4). The data from rabbits were also successfully fitted to a linear two-compartment open model with one distribution phase and one elimination phase (Table 5). The distribution and elimination half-lives were 0.5 and 33.8 h, respectively.

4. Discussion and conclusion

HPLC–ED is a popular method for the analysis of morphine in biological samples. Many published papers describe the need for multiple extraction and complex chromatographic systems to ensure reproducibility and resolution [3,14,15]. In the current study, we have reported a simple, rapid, sensitive, specific and accurate method for the determination of morphine.

Table 5
Pharmacokinetic parameters in three rabbits after single intravenous injection of morphine 3 mg/kg

Number	A (ng/ml)	B (ng/ml)	α (1/h)	β (1/h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$AUC_{0-\infty}$ (h ng/ml)	CL_t (l/h/kg)
1	206	35.9	1.21	0.08	0.57	8.48	609.9	4.92
2	432	9.1	1.36	0.02	0.51	32.0	735.3	4.08
3	283	6.7	1.65	0.01	0.42	60.1	763.2	3.93
Mean	307	17.2	1.41	0.04	0.50	33.8	702.8	4.31
S.D.	115	16.2	0.22	0.04	0.08	26.3	81.6	0.53

[a] Equation: plasma concentration (C_p) = $Ae^{-\alpha t} + Be^{-\beta t}$; A, B: intercept; α, β: the first-order rate constants for the central and tissue compartments; $t_{1/2}$ = half-life of the first-order rate constant; $AUC_{0-\infty}$: area under the time–concentration graph from time zero to time infinity; CL_t = total plasma clearance.

Morphine is an alkaloid that contains a tertiary amine as part of a ring structure and a phenolic group at C-3 (plus a hydroxyl group at C-6) (Fig. 1). The pK_a values of the ionizable groups of morphine are 7.9 and 9.9, respectively; therefore, morphine has the lowest net charge and the best solubility in organic solvents at pH 8.9 which is at the mid-point between the two pK_a values [1–3]. Consequently, this pH was chosen for the liquid–liquid extraction [2,3]. Similarly to other basic compounds, morphine tends to adsorb onto the surface of glassware, particularly when its concentration is very low [4]. Therefore, the glassware used in the current study was silanized. Before silanization, we found that the recovery of morphine after extraction was very unstable, around 30%. After silanization, the recovery of morphine improved to a level of 86–90%.

As reported by several authors [25] a silica column with an aqueous eluent was used in our study for the chromatography of morphine. This method has been used in our laboratory for the determination of opioids for about 8 years [19,20,26]. In order to protect the column and to improve its lifetime a precolumn, packed with the same material as the main column, was used and the mobile phase was saturated with silica powder. Adopting these precautions, the column was maintained in good conditions for years. Additionally, we observed a good reproducibility among different batches of the silica column.

Neither M3G nor M6G were detectable in the current study. While M3G lacking the phenolic hydroxyl is 'per se' not oxidizable at the used potential, M6G should be detected by ED. However, M3G and M6G are more polar than their parent drug and will not be extracted at pH 8.9.

In conclusion, compared with the previously reported HPLC methods for quantitation of morphine, this assay offers two advantages, a one-step extraction procedure and a very low level of quantitation (100 pg/ml).

Acknowledgements

We thank Ms. May-Jane Chen for technical assistance. This work was supported in part by the National Science Council (NSC 87-2314-B-016-019) and in part by the Department of Health, Executive Yuan (DOH85-TD-125), Taiwan.

References

[1] J.H. Jaffe, W.R. Martin, in: A.G. Gilman, T.W. Rall, A.S. Nies, P. Taylor (Editors), The Pharmacological Basis of Therapeutics 3rd ed., Pergamon Press, New York, 1996, p. 485.
[2] F. Tagliaro, D. Franchi, R. Dorizzi, M. Marigo, J. Chromatogr. 488 (1989) 215.
[3] M.K. Leung, Adv. Neuroimmunol. 4 (1994) 93.
[4] P.H. Jorden, J.P. Hart, Analyst 116 (1991) 991.
[5] R. Wasels, F. Belleville, J. Chromatogr. A 674 (1994) 225.
[6] P. Horak, F. Haberman, S. Spector, Life Sci. 52 (1993) 255.
[7] G.W. Hanks, M. Hanna, I. Finlay, D.J. Radstone, T. Keeble, J. Pain Sympton Manag. 10 (1995) 6.
[8] R.F. Kaiko, R.D. Fitzmartin, G.B. Thomas, P.D. Goldenheim, Pharmacotherapy 12 (1992) 107.
[9] M.P. Thirlwell, P.A. Sloan, J.A. Maroun, G.J. Boos, J.G. Besner, J.H. Stewart, B.M. Mount, Cancer 63 (1989) 2275.
[10] B. Fryirs, M. Dawson, L.E. Mather, J. Chromatogr. B 693 (1997) 51.
[11] M.J. Bogusz, R.D. Maier, K.H. Schiwy-Bochat, U. Kohls, J. Chromatogr. B 683 (1996) 177.
[12] R.W. Milne, R.L. Nation, G.D. Reynolds, A.A. Somogyi, J.T.V. Crugten, J. Chromatogr. 565 (1991) 457.
[13] P.A. Glare, T.D. Walsh, C.E. Pippenger, Ther. Drug Monit. 13 (1991) 226.
[14] A.W.E. Wright, J.A. Watt, M. Kennedy, T. Cramond, M.T. Smith, Ther. Drug Monit. 16 (1994) 200.
[15] F. Tagliaro, G. Carli, R. Dorizzi, M. Marigo, J. Chromatogr. 507 (1990) 253.
[16] H. Derendorf, M. Kaltenbach, J. Pharm. Sci. 75 (1986) 1198.
[17] A.I. Bouquillon, D. Freeman, D.E. Moulin, J. Chromatogr. 577 (1992) 354.
[18] Y. Liu, T.V. Bilfinger, G.B. Stefano, Life Sci. 60 (1997) 237.
[19] S.T. Ho, J.J. Wang, W. Ho, Y.O.P. Hu, J. Chromatogr. 570 (1991) 339.
[20] S.T. Ho, J.J. Wang, O.Y.P. Hu, P.S. Chiang, S.C. Lee, J. Chromatogr. B 678 (1996) 289.
[21] C.M. Metzler, D.L. Weiner, PCNONLIN User's Guide, Statistical Consultants, Lexington, KT, 1989.
[22] A.J. Sedman, J.G. Wagner, J. Pharm. Sci. 65 (1976) 1006.
[23] P.G. Welling, Drug Devel. Indus. Pharm. 9 (1983) 1185.
[24] L. Shargel, A.B.C. Yu (Editors), Applied Biopharmaceutics and Pharmacokinetics, Prentice-Hall, New York, 3rd ed., Ch. 5, 9, 20, 1993.
[25] S.R. Binder, in: J.A. Adamovics (Editor), Analysis of Addictive and Misused Drugs, Marcel Dekker, New York, 1995, p. 133.
[26] S.T. Ho, J.J. Wang, O.Y.P. Hu, T.M. Hu, Biopharm. Drug Dispos. 16 (1995) 695.