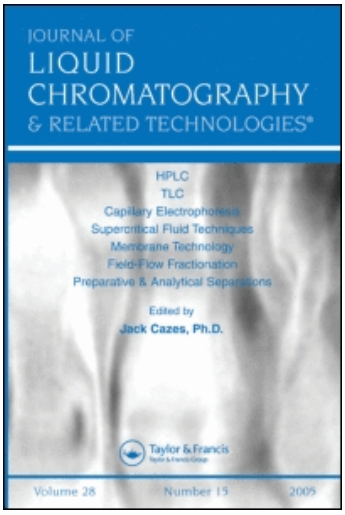

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QUANTIFICATION OF PENTAZOCINE IN HUMAN PLASMA BY HPLC WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

A rapid and sensitive high performance liquid chromatographic method with electrochemical detector was described to measure pentazocine concentrations in plasma. The separation of pentazocine and an internal standard, levallorphan, from interfering compound in plasma was achieved by reversed phase phenyl column chromatography in combination with a solid extraction cartridge as clean-up. Analytical recoveries for pentazocine and levallorphan were determined as 88.6 % and 92.8 %, respectively. The detection limit was determined as 500 pg/ml plasma. This method has been successfully applied to the pharmacokinetic study of pentazocine in healthy male volunteers dosed rectum at 50 mg and plasma levels monitored up to 24 h after dosing.

INTRODUCTION

Pentazocine, butorphanol and buprenorphine are non-narcotic analogue of morphine. Pharmacologically, these drugs are thought to provide equivalent analgesia to morphine (when adjusted for potency), and widely used in the management of patients with post-operative pain or initial carcinogenic pain (1). Though pentazocine has the widest therapeutic range in these drugs, repetitive injection (per 2-3 h) is necessary for duration of sedation because it is only commercially available as an injection for intramuscular or intravenous administration. To prolong its action, suppositories containing 50 mg of pentazocine have been prepared with esterified fatty acid (oil type) and polyethylene glycol (water soluble type) and studied clinically at the Social Insurance Chukyo Hospital in Nagoya, Japan. In order to evaluate efficacy of the suppositories the accurate measurement of pentazocine in plasma was required.

The quantification of pentazocine has been achieved by gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC). These GC (2), GC/MS (3) and HPLC (4) with derivatization methods are time-consuming, because they need a complicated procedure. HPLC with ultraviolet (UV)-detection did not have enough sensitivity to apply to routine analysis. Shibanoki *et al.* (5) and Moeller *et al.* (6) have reported highly sensitive quantification methods of pentazocine using HPLC with electrochemical detection (ECD) and fluorometric detection, respectively. Although both methods shows the almost same detection limit, ECD was chosen in the study, because it is highly sensitive and specific for phenolic hydroxy group that is a common moiety of benzomorphane-type compounds. Additionally, it was expected that ECD is able to detect not only pentazocine but also its metabolites and related compounds. However, these HPLC methods have a drawback in

separation that octadecyl silanized (C18) silica gel show poor retention for pentazocine and levallorphan as an internal standard. Probably, the retention behavior would cause some problems such as poor resolution of its metabolites and interfering peaks from plasma. Although the two groups used liquid-liquid extraction for clean-up (5, 6), it is not always suitable for routine analysis at a hospital, because a large number of samples must be treated and a large amount of inflammable organic solvents must be used.

In order to establish a simple, rapid and reliable HPLC with ECD for analysis of pentazocine, which is available at even an usual hospital, we have mainly examined the following three points.

1) improvement of sensitively by ECD

2) optimization of separation conditions in HPLC

3) establishment of clean-up using a solid-phase extraction

Finally, the established method was applied to a pharmacokinetic study of pentazocine using healthy male volunteers.

EXPERIMENTAL

Materials

Methanol, acetonitrile, sodium dihydrogen phasphate, disodium hydrogen phasphate, citric acid, trisodium citrate and trifluoro acetic acid were analytical grade materials. Water was purified from a Millipore Milli-Q SP system (Bedford, MA, U.S.A.). Cosmosil 5Ph, 5C18, and 5CN-R (Nacalai tesque, Kyoto, Japan) were used as HPLC analytical columns. Bond Elut Ph and C18 cartridges were provided by Analytichem International (Harbor city, CA, U.S.A.). Pentazocine as a free base was a gift from Sankyo Co., Ltd. (Tokyo, Japan). The internal

standard, levallorphan tartarate was supplied by Takeda Chemical Industries Co., Ltd. (Osaka, Japan).

Chromatographic system

The HPLC system consisted of a Tosoh Model CCPE pump (Tokyo, Japan) equipped with a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector with 100 µl loop, a Jasco Model 840-EC electrochemical detector (Tokyo Japan), coupled in series with a Tosoh Model UV-8000 ultraviolet detector and a Shimadzu Model CTO-6A column oven (Kyoto, Japan). The separation was performed on a Nacalai Cosmosil 5Ph (5 µm, 4.6 mmI.D. x 250 mm, Tokyo, Japan) with acetonitrile-0.1M phosphate buffer (pH 6.0) (30:70) as a mobile phase at 35 °C. The electrochemical potential was set at +900 mV *versus* a saturated caromel reference electrode and ultraviolet detector was operated at 278 nm.

Extraction and clean-up procedure

One ml aliquot of plasma was diluted by adding 2 ml of water and 100 µl of 0.5 mg/ml levallorphan solution. The solution was applied to Bond Elut Ph cartridge which had been previously washed with 6 ml of methanol and 12 ml of water. The cartridge was washed with 6 ml of methanol-water (60:40) to eliminate most of plasma constituents. The desired compounds were then eluted with 4 ml of methanol. The eluate was concentration under reduced pressure, and the residure was dissolved in 200 µl of the mobile phase prior to injection of 50 µl into the HPLC column.

Application

Each blood sample (4.5 ml) was collected from healthy male volunteers (Table 1) after insert of pentazocine (50 mg) suppository with a Nipro model NT-

TABLE 1

Characteristics of healthy male volunteers (mean ± S.D.)

Group	Age (years)	Weight (kg)
Oil type (n=9)	30.3 ± 6.7	64.4 ± 3.3
Water soluble type (n=11)	29.6 ± 6.3	65.7 ± 5.5

CS0457 tube (Tokyo, Japan) containing 3.8 % sodium citrate at 11 points (0, 20, 40, 60, 120, 180, 240, 360, 480 720 and 1440 min). The samples were centrifuged at 2,000 rpm for 5 min and then 1 ml of plasma was dispensed into a test tube. The plasmas were treated in the same manner as described above.

Pharmacokinetic analysis

The plasma concentration-time data for pentazocine were fitted to an one-compartment open model by weighted least-squares analysis with the MULTI pharmacokinetic curve-fitting program (7). The area under the curve (AUC) for plasma pentazocine concentration was determined by the linear trapezoidal rule with extrapolation to infinity.

RESULTS AND DISCUSSIONS

Optimization of chromatographic separation

Shibanoki *et al.* used acetonitrile-0.05M citrate buffer (pH 4.0)-pyridine (69.8 :30:0.2) as a mobile phase to separate pentazocine and levallorphan by HPLC/ECD. However, their retention times were considerably short. Probably, the separation

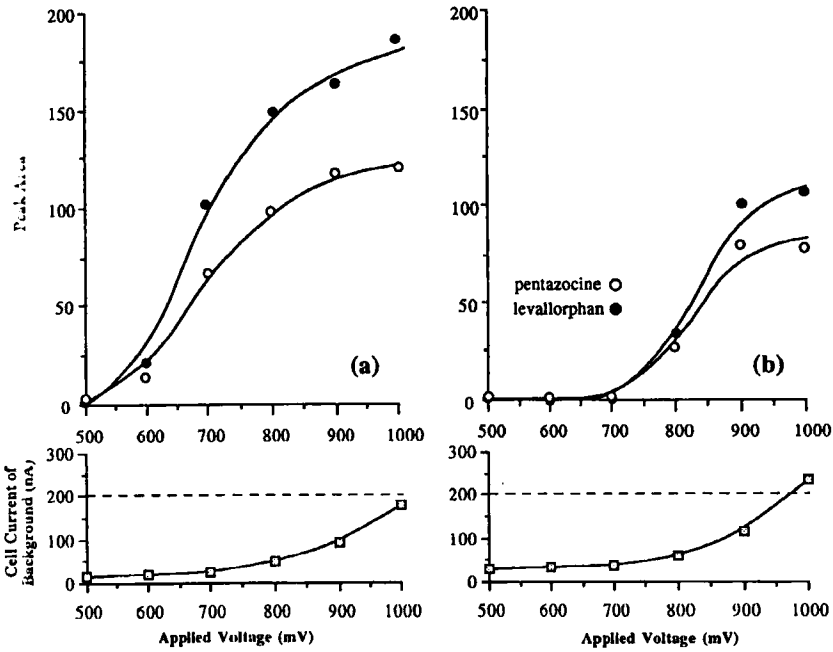

Fig. 1. Hydrodynamic voltammograms and relationship between cell current of background and applied voltage profile in (a) acetonitrile-phosphate buffer (pH 6.0) (30:70) and (b) acetonitrile-citrate buffer (pH 5.0) (35:65).

conditions would be not appropriate for pentazocine, levallorphan and more polar compound such as metabolites of pentazocine. Although, an addition of water or buffer to a mobile phase causes a retention time to lengthen in reversed phase HPLC, an electrochemical responce resulted in lowering, which depends on the applied voltage and cell current of back ground in an electrochemical detector. In order to obtain high sensitivity of pentazocine and levallorphan by ECD, citrate (pH 5.0) and phosphate (pH 6.0) buffers were compared. The relationships between cell current of background and applied voltage curve and between cell current and voltage curve (hydrodynamic voltammograms) of pentazocine and levallorphan in

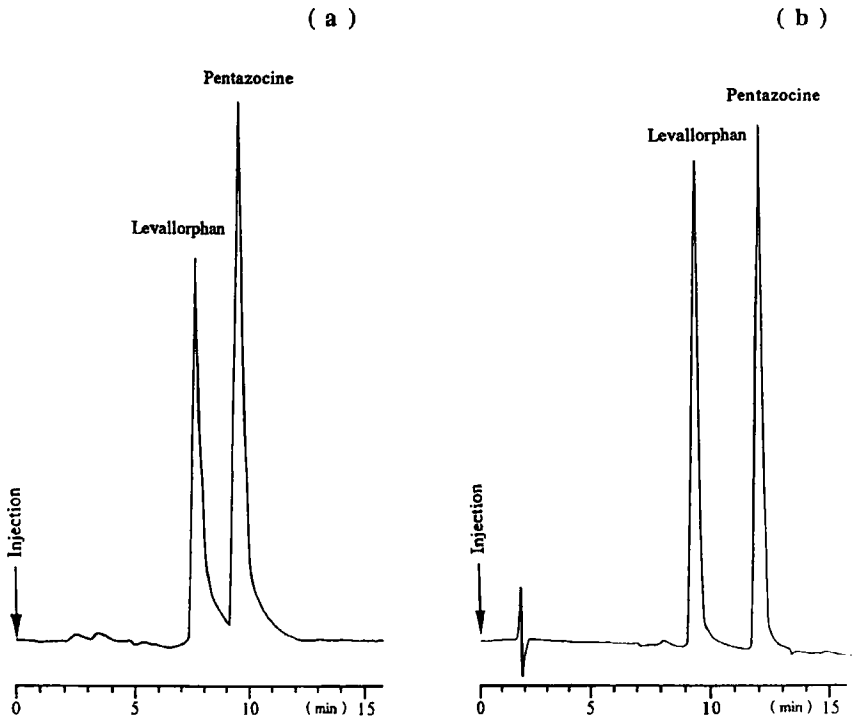

Fig. 2. High performance liquid chromatograms of pentazocine (10 ng) and levallorphan (10 ng) using (a) C18 column and (b) Ph column, and acetonitrile-0.1M phosphate buffer (pH 6.0) (30:70) as a mobile phase .

two mobile phase systems are shown in Fig. 1. The hydrodynamic voltammograms show clearly that the electrochemical response in phosphate buffer is twice as sensitive as that in citrate buffer. In phosphate buffer their electrochemical responses did not reach a plateau at +1000 mV and the cell current of background become more than 200 nA. Since cell current of background above 200 nA causes instability of the detector, the applied voltage was set +900 mV.

Fig. 2a shows the high performance liquid chromatogram of both compounds using a C18 column and acetonitrile-0.1 M phosphate (pH 6.0) (30:70)

TABLE 2

Comparison of chromatographic parameters for separation of pentazocine and levallorphan by three columns using 0.1 M acetonitrile-phosphate buffer (pH 6.0) (30:70) as a mobile phase

		C18	Ph	CN-R
t_R	pentazocine	9'12"	11'36"	11'24"
	levallorphan	7'24"	9'00"	9'00"
Rs*		2.25	4.73	4.00
As**	pentazocine	3.50	1.70	2.10
	levallorphan	4.00	2.00	1.75

*$Rs = (t_1-t_2) / (w_1-w_2)$, where w_1 and w_2 are the corresponding peak widths at the base line

**As is the ratio of the rear to the front lengths of the peak along a line parallel to 10% of its height distant from its base line.

as a mobile phase. Although their retention times are almost appropriate, both peaks show serious tailing, suggesting that C18 column is not suitable. We compared C18 column with Ph (phenyl) and CN-R (cyano) columns using the following three parameters retention times (t_R), resolution factor (Rs,) and asymmetry factor (As). Table 2 shows three chromatographic parameters for the separation of both compounds using the three columns. The replacement of C18 column by Ph and CN columns makes their retention time longer and improves their peak shapes. Both compounds are separated with better resolution with Ph column than that with CN column. The typical separation is illustrated in Fig. 4b under the following optimized conditions; column, Cosmosil 5Ph (4.6 mmI.D. x 250 mm), mobile phase: acetonitrile-0.1 M phosphate buffer (pH 6.0) (30:70), flow rate: 1.0 ml/min, column oven temp.: 35 °C, ECD applied voltage: +900 mV *versus*

a saturated caromel electrode. The detection limit was found to be 500 pg (S/N 10).

Extraction procedure

In the case of analysis of a small amount of analyte in complicated matrix such as plasma, extraction and clean-up that extract completely and concentrate effectively it, depend on its total recovery. Although the liquid-liquid extraction gave satisfactory recovery (5, 6), the method is time-consuming and Moelloer's method using ice-cooled diethyl ether is not suitable for treatment of many samples and in an usual hospital.

The chromatographic behavior of pentazocine and levallorphan mentioned aboved reminded us to use solid phase extraction using reversed phase cartridges for extraction and clean-up steps. As shown in the previous experiment pentazocine and levallorphan were expectedly retained more strongly on Ph cartridge than on C18 cartridge and the former was used in the subsequent work. When the analytes in water were charged on it, they were not eluted with methanol alone but were eluted with 0.01N TFA-methanol, whereas both compounds in plasma solution were easily eluted with methanol alone, probably due to influence by some contaminants in the matrix. Since the desired methanol fraction contained many unnecessary impurities from plasma, washing was evaluated by changing solvent containing different ratio of methanol-water. It was found that washing with methanol-water (60:40) eliminates efficiently contaminants from plasma without elution of pentazocine and levallorphan from Ph cartridge. From these results 6 ml of methanol-water (60:40) and 12 ml of methanol were used as washing and eluting solvents, respectively in the subsequent experiment.

The limit of quantification was 3.0 ng/ml of plasma, and the recovery values of pentazocine and levallorphan in plasma are 88.6 ± 3.8 % (cv 4.3, n=5) and 92.8

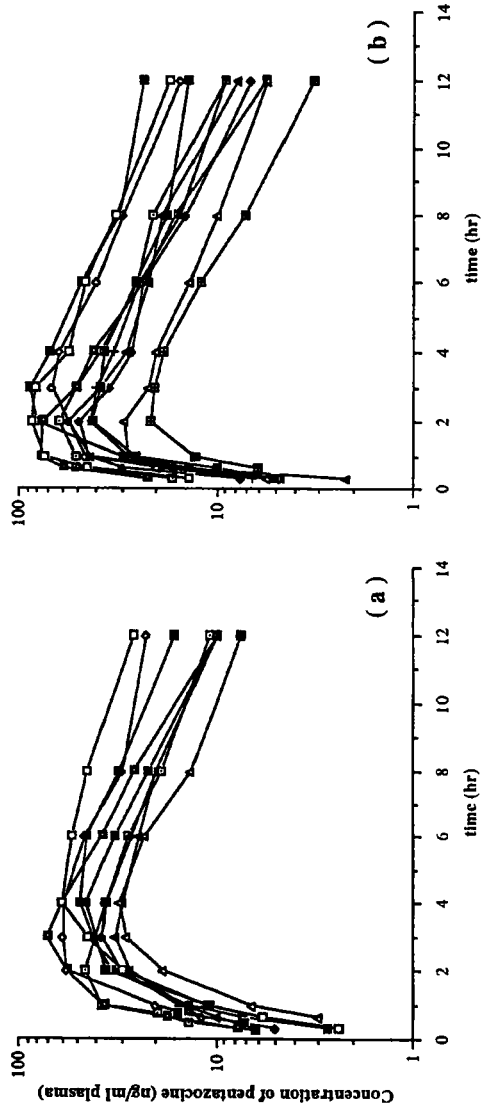

Fig. 3. Concentration of pentazocine in plasma from healthy male volunteers administrated (a) oil type suppository and (b) water soluble type suppository.

TABLE 3

Comparison of pharmacokinetic parameters for pentazocine in plasma, blood or serum by several analitical methods

Method	Pharmacokinetic parameter			
	Ke (hr^{-1})	Vd (l)	Cl (l/hr)	$T_{1/2}$ (hr)
Fluorometric (8)	-	-	-	2.1
Gas Chromatography (9)	0.16 ± 0.02	507 ± 41	79 ± 8.3	4.6 ± 0.41
Mass fragmentgraphy (3)*	0.309**	208***	-	2.3****
Radio-receptor assay (10)	-	251 - 548	63.6 - 102	1.5 - 6.5
HPLC (present method)				
oil type	0.225 ± 0.056	457 ± 193	102.8 ± 10.8	3.1 ± 0.6
water soluble type	0.229 ± 0.044	413 ± 172	94.6 ± 7.6	3.0 ± 0.5

* calculated withthe two-compartment open model

** the slope of β phase was calculated with the mean rate constant (k12, K21 and Ke).

*** the total volume of distribution (sum of volume of central and peripheral compartment).

**** the β phase half-life.

± 1.6 % (cv 1.7, n=5), respectively. The peak area ratio of pentazocine were lineally related (Y=0.0628 X - 0.0168, r= 0.998) to the amount of pentazocine added to blank plasma in the range of 0.5 - 200 ng/ml .

Application

This method has been successfully applied to a pharmacokinetic study of pentazocine in healthy male volunteers dosed rectum at 50 mg and its plasma levels were monitored up at 12 h after dosing (Fig. 3a and b). Using oil type pentazocine suppository average area under curve (AUC), average absorption rate (Ka) and average excretion rate (Ke) and apparent volume of distribution (Vd) were 445± 187 (ng/ml·h), 0.349±0.110 (h^{-1}), 0.225±0.056 (h^{-1}) and 7.1 ± 3.0 (l/kg),

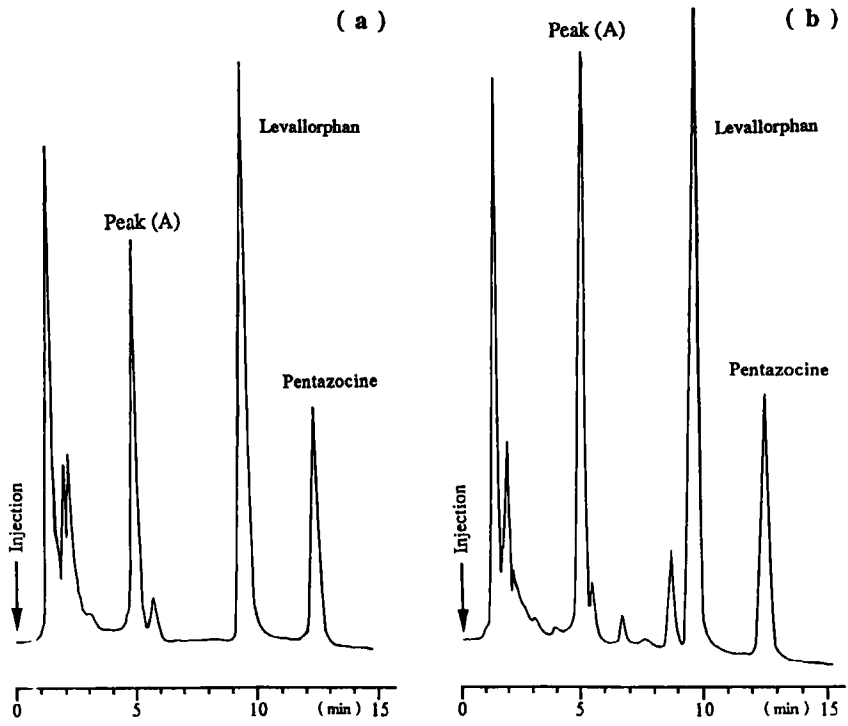

Fig. 4. High performance liquid chromatograms of the desired fractions from plasma of a volunteer (a) and a patient (b) at 6 hr after incert of an oil type suppository.

respectively. In the case of water soluble type , AUC, Ka, Ke and Vd were 413± 172 (ng/ml·h), 0.734±0.341 (h^{-1}), 0.229±0.044 (h^{-1}) and 7.0 ± 3.3 (l/kg), respectively. Ka of water soluble type suppository was twice as large as that of oil type one. The pharmacokinetic parameters measured in our study are compared with those by the other assays previously reported in the literatures (TABLE 3). All parameters in this study were in the range of the previous data. The typical chromatograms of a volunteer and a patient are illustrated in Fig. 4a and b, respectively. The peak (A) seems to be a peak of metabolites of pentazocine. We

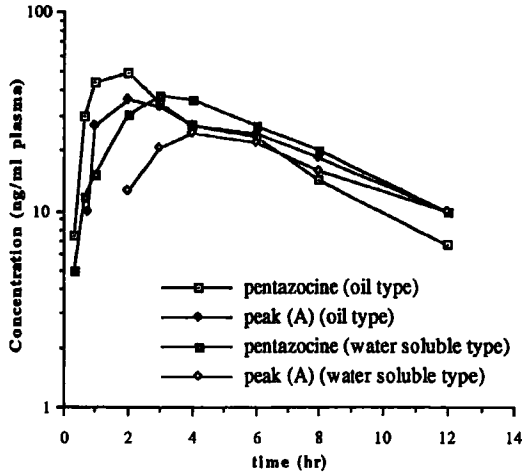

Fig. 5. Concentration of pentazocine and peak (A) in plasma from a healthy male volunteer administrated an oil type and water soluble type suppositories.

have confirmed that peak (A) is not *cis*- and *trans*-hydroxymethyl derivatives of pentazocine, which are two of the methabolites (11). Concentration of pentazocine and peak (A) in plasma from a health male volunteer administration oil type and water soluble type suppositories was illustrated in Fig. 5. In the case of a patient who take another therapeutic drug, pentazocine and levallorphan were able to be analyzed without interfering (Fig 4b).

In summary, we have developed a rapid, simple and sensitive analytical technique for pentazocine in plasma, and have demonstrated its application in a pharmacokinetic study of pentazocine suppository. Use of a Ph column in combination with ECD using acetonitrile-phosphate buffer as a mobile phase increased further sensitivity and reliability of the separation. Replacement of liquid-liquid extraction by a Ph cartridge as clean-up permitted to establish a simple

and rapid quantification method of pentazocine , which is available at even an usual hospital.

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