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Development and Validation of a Stability Indicating HPLC Method for the Determination of Buprenorphine in Transdermal Patch

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ABSTRACT

A rapid and sensitive stability indicating HPLC method has been developed and validated for the determination of buprenorphine (BPN) in transdermal patch. Chromatographic separation was achieved isocratically on an XBridge^{TM} Shield RP18 column with a mobile phase of acetonitrile / 0.063 M ammonium bicarbonate buffer (pH 9.5) (58:42, v/v) at the flow rate of 1.5 mL/min with UV absorbance monitoring at 230 nm. The system performance was evaluated and the result showed that BPN and degradation products were separated. Buprenorphine was subjected to neutral, acidic and basic hydrolysis as well as chemical oxidation to evaluate the specificity. The calibration curve of buprenorphine was linear in the range of 30~70 μg/mL (r = 0.9999, n = 5). The values of RSD (%) for the intra-day and inter-day precision ranged from 0.04 to 0.22 and 0.65 to 0.88%, respectively. The average of the recovery percentage ranged from 98.86 to 99.36%. The detection limit (DL) and quantitation limit (QL) for buprenorphine were 0.008 and 0.024 μg/mL, separately. The robustness of this method was also evaluated on the small fluctuations of pH in the mobile phase, the mobile phase compositions, and the flow rate. The results of stability studies showed the hydrolysis reaction of buprenorphine followed zero-order kinetics model in acidic and basic environment and followed first-order kinetics model in the presence of hydrogen peroxide. This analytical method was successfully applied to the determination of buprenorphine in transdermal patch and can be used for routine quality control work.

Key words: HPLC, validation, stability indicating, buprenorphine, transdermal patch

INTRODUCTION

Buprenorphine (Figure 1) is a semisynthetic opioid derivative, closely related to morphine and congener alkaloids, which is obtained from thebaine after a seven-step chemical process[(1)]. Buprenorphine has been used to control cancer pain and can be given by several administration routes. Parenteral and sublingual preparations were initially used[(2)]. In recent years, the formulation of buprenorphine in transdermal matrix patch has been successfully developed[(3)].

An opioid derivative could form an ion pair between its basic nitrogen and a negatively charged group, such as from sodium 1-heptanesulfonate. Thus, it could be determined by using the ion-pair reversed-phase HPLC. However, the retention time of buprenorphine obtained in USP 30[(4)] and EP 5.0[(5)] are long and the sensitivity of the recommended methods are unsatisfied.

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The XBridge^{TM} column was designed to overcome some limitations of classical silica based reversed-phase HPLC columns with a wider pH limit of 1-12. Buprenorphine is a weak base with a pKa of 8.24 and therefore alkaline environment will favor the unionized form of the drug and offer a symmetrical peak shape[(6,7)].

The objective of this study was to develop an effective and sensitive stability indicating method for transdermal buprenorphine patch by using the XBridge^{TM} column and HPLC-UV detector and to validate this method according to the compendious guidelines[(8,9)].

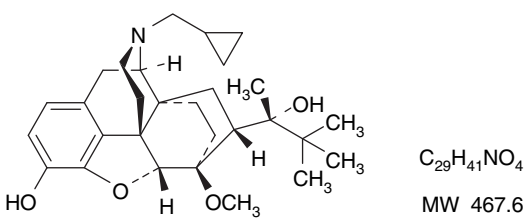

Figure 1. Structure of buprenorphine[(5)].

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MATERIALS AND METHODS[8-12]

I. *Materials*

Buprenorphine, 21-cyclopropyl-7α-[(S)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-endo-ethano-6,7,8,14-tetrahydrooripavine, was purchased from Tasmanian Alkaloid (Australia). The USP reference standard of buprenorphine related compound A (impurity), 21-[3-(1-propenyl)]-7α-[(S)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-endo-ethano-6,7,8,14-tetrahydrooripavine, was purchased from the US Pharmacopeial Convention[4].

Polystyrene-*block*-polyisoprene-*bolck*-polystyrene (SIS, 17 wt.% styrene), rosin ester (KE-311), and paraffin oil (puriss., meets analytical specification of Ph. Eur., BP, viscous liquid) were purchased from Sigma-Aldrich Co., Arakawa Chemical Co., and Riedel-de Haën Co., respectively. All other solvents and reagents were of analytical or HPLC grade.

II. *HPLC Instrumentation and Chromatographic Conditions*

The analyses of the samples in this study were performed on a Hewlett-Packard 1100 HPLC system (AGILENT Technologies) which is equipped with a G1311A quaternary pump, a G1313A automatic injector, a G1314A photodiode array detector, a Chemstation version A.07 data acquisition software, and a G1316A column oven.

The chromatographic conditions were as follows: separations were carried out at 30°C on an XBridge™ Shield RP18 column (4.6 × 75 mm, 2.5 μm; Waters), the mobile phase consisted of acetonitrile / 0.063 M ammonium bicarbonate buffer (pH 9.5) (58:42, v/v) at the flow rate of 1.5 mL/min. The mobile phase was degassed in an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.22 μm filter prior to use. The injection volume was 20 μL for each analysis. The chromatography was monitored by the absorbance at 230 nm.

III. *Preparation of Stock and Standard Solution*

Stock solution of buprenorphine (0.5 mg/mL) was prepared in methanol. Standard solution of buprenorphine (50 μg/mL) was prepared by diluting the stock solution of buprenorphine with the mobile phase. The peak shape of standard solution of buprenorphine was measured by the photodiode array detector at wavelengths of 210, 230, 254, and 288 nm.

IV. *System Suitability*

Stock solution of buprenorphine impurity (50 μg/mL) was prepared in methanol. An aliquot of 5 mL stock solutions of buprenorphine and its impurity were individually placed into a 50 mL volumetric flask and made to volume with the mobile phase for system suitability study. This solution was analyzed for six times to obtain the parameters of system suitability.

V. *Specificity*

Sample solution of buprenorphine (50 μg/mL) was used to evaluate the specificity of this analytical method under the neutral, acidic, and basic conditions, while stock solution of buprenorphine was used to evaluate the specificity under the chemical oxidative condition at 80°C over certain period of time. The media and the reaction times were as follows: (a) 0.1 M pH 7.0 phosphate buffer solution for 120 hours, (b) 1.0 M HCl solution for 6 hours, (c) 1.0 M NaOH solution for 24 hours, and (d) 10% H_2O_2 solution for 2 hours. All samples were neutralized and diluted before analysis. Each solution was analyzed in duplicate.

VI. *Linearity and Range*

By appropriate dilution with the mobile phase, five different concentrations of the standard solutions of buprenorphine from 30 to 70 μg/mL were prepared. The solutions were measured, separately, for five times according to the chromatographic conditions previously given.

VII. *Precision*

The intra-day and inter-day precision were determined by analyzing the samples of buprenorphine at the concentrations of 30, 50, and 70 μg/mL. Each sample was analyzed for five times on the same day. They were studied over three consecutive days and each intra-day analysis was performed by different operators.

VIII. *Accuracy*

The adhesive solution was made by adding SIS, rosin ester and paraffin oil in a ratio of 45:55:10 into coating solvent ethyl acetate and mixing on a reciprocal shaker at 250 rpm at room temperature[13]. Three adhesive solutions containing 1.5, 2.5, and 3.5 mg of buprenorphine, individually, were coated on the release liner and the adhesive films were allowed to dry at 50°C for 20 minutes. They were stored at room temperature for 10 minutes. The backing sheets were then placed on the dry adhesive to prepare the transdermal buprenorphine patches.

Each patch was transferred into a 50 mL volumetric flask and made to volume with the mobile phase. They were sonicated at the 60°C for 1 hour. All of them were filtered through a 0.22 μm filters. Each sample was analyzed for five times.

IX. *Detection Limit and Quantitation Limit*

By appropriate dilution of the stock solution of buprenorphine with the mobile phase, three standard

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solutions of 0.25, 0.5, and 1 μg/mL were prepared. Each standard solution was assayed for three times. Linear regression analysis was performed to obtain the regression equation. The detection limit (DL) and the quantitation limit (QL) were calculated from DL = 3.3 σ/S , and QL = 10 σ/S

Where σ is the standard deviation of the Y-intercept and S is the slope of the calibration curve.

X. *Robustness*

The influence of deliberate small variations of the factors in the determination of buprenorphine were examined in order to estimate the robustness of the proposed method. The factors to be examined were: pH in the mobile phase, the mobile phase compositions, and the flow rate. One factor was changed at a time and the analytical values were examined accordingly[14].

XI. *Stability Studies*

(I) *Acidic Stability*

A sealed sample bottle contained 180 mL 0.1 M HCl and 20 mL stock solution of buprenorphine were mixed in a sealed sample bottle and stored at 60°C in the oven. An aliquot of 5 mL was taken out every day and kept in a freezer.

(II) *Basic Sstability*

A sealed sample bottle contained 180 mL 0.1 M NaOH and 20 mL stock solution of buprenorphine were mixed in a sealed sample bottle and stored at 80°C in the oven. An aliquot of 5 mL was taken out every 4 hours and kept in a freezer.

(III) *Chemical Oxidative Stability*

A sealed sample bottle contained 200 mL 10% H_2O_2 and 200 mL stock solution of buprenorphine were mixed in a sealed sample bottle and stored at 60°C in the oven. An aliquot of 5 mL was taken out every half an hour and/or one hour and kept in a freezer.

Each sample was analyzed for three times in order to obtain the kinetic profiles.

RESULTS AND DISCUSSION

I. *Development of the HPLC Method*

The chromatograms and absorption spectrum of buprenorphine in this study were shown in Figure 2. The retention time of buprenorphine was about 7.8 minutes. The maximal absorption was at the 210 nm. The wavelength of the detection adopted in USP 30 and EP 5.0 was at the 288 nm. When the standard solution of buprenorphine was measured at 210, 230, 254, and 288 nm individually, all the peak shapes detected at different wavelengths were acceptable (see Figure 2(B)). Finally, the adopted wavelength was set at 230 nm as the sensitivity was concerned.

II. *System Suitability*

The parameters of system suitability of buprenorphine and its impurity showed that the peaks were completely separated. The system suitability data were listed in Table 1 and the chromatogram of buprenorphine and its impurity was shown in Figure 3.

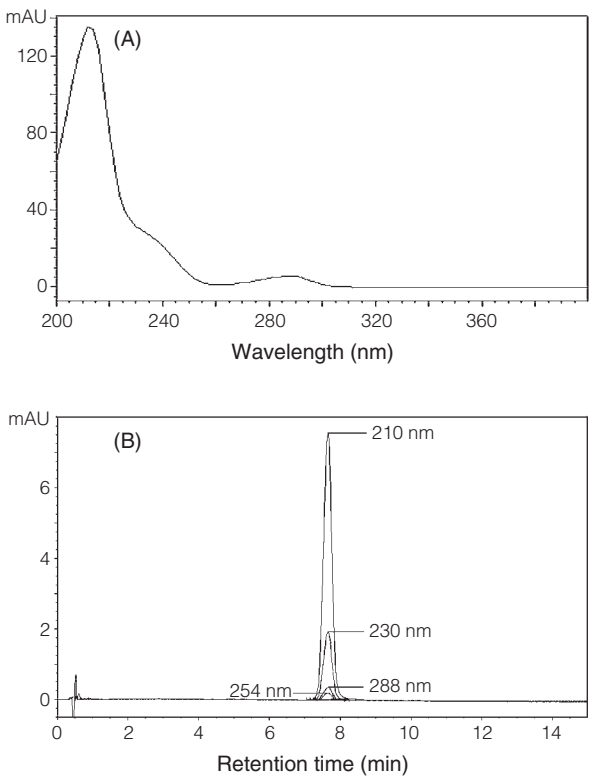

Figure 2. (A) Absorption spectrum of buprenorphine in the mobile phase. (B) The standard solution of buprenorphine was detected at the wavelengths of 210, 230, 254, and 288 nm, simultaneously.

Table 1. The system suitability evaluation of the HPLC method developed

	buprenorphine	buprenorphine impurity
Retention time (min)	7.80	8.80
K' (capacity factor)	16.20	18.03
N (theoretical plate)	9821	9764
T (tailing factor)	1.18	1.10
Rs (resolution)	2.50	

III. *Specificity*

The percentage of remaining concentration of buprenorphine in specificity studies were given in Table 2.

Chromatograms of specificity studies were given in Figure 4. When the stock solution of buprenorphine was exposed to neutral hydrolysis, no decomposition was observed which indicated it was quite stable under the neutral hydrolysis condition (Figure 4 (A)). However, when the stock solutions of buprenorphine were exposed to acidic, basic, or oxidative media as described in Table 2, the percentage of remaining concentration were about 43, 75, and 30%, respectively (Figure 4 (B)-(D)). The other decomposed products were completely separated from buprenorphine indicating that the developed method could be useful to separate buprenorphine from its degradation products.

IV. *Linearity and Range*

Linear relationship was observed in the calibration curve in the range of 30~70 μg/mL. The regression equation was found as y = 13371x - 4481.6 (r = 0.9999, n = 5), where y is the peak area of buprenorphine and x is the concentration of the measured solution in μg/mL.

V. *Precision*

The intra-day (n = 5) and inter-day (n = 15, three different days) precision were expressed as relative standard deviation (RSD). The values of RSD for the intra-day and inter-day precision were ranged from 0.04 to 0.22% and 0.65 to 0.88%, respectively. The results revealed good precision for the proposed analytical method (see Table 3).

VI. *Accuracy*

The recovery of the transdermal buprenorphine patches were calculated from: Recovery (%) = [Ct / Ca] × 100

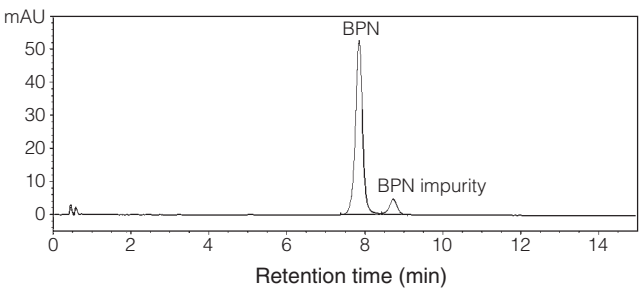

Figure 3. The chromatogram of buprenorphine and its impurity.

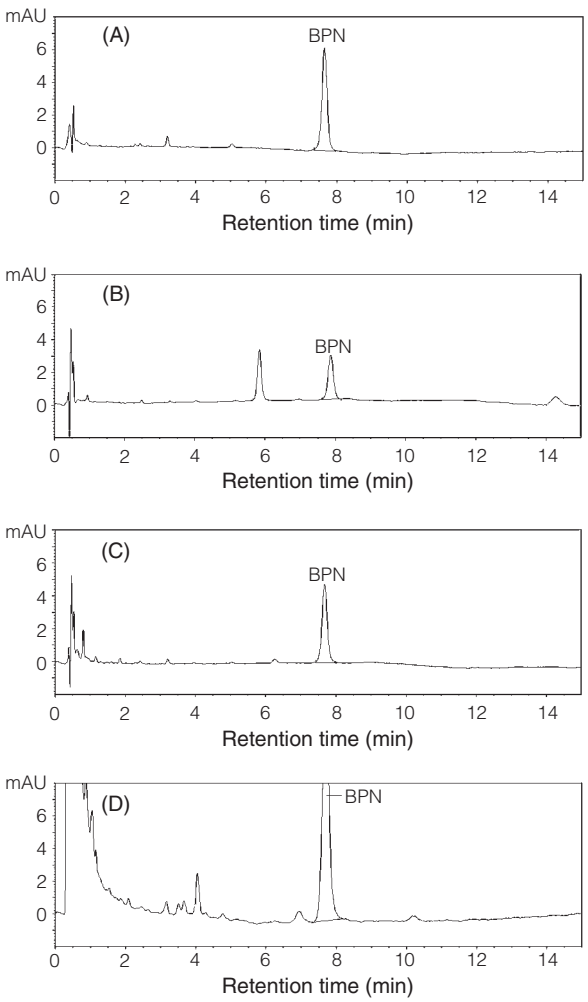

Figure 4. Chromatograms corresponding to buprenorphine solution underwent (A) neutral hydrolysis (B) acidic hydrolysis (C) basic hydrolysis (D) chemical oxidation.

Table 2. Remaining concentration of buprenorphine in specificity studies

Condition		Time (hr)	The percentage of remaining concentration (%)
Neutral hydrolysis	0.1 M pH 7.0 phosphate buffer, 80°C	120	98.97
Acidic hydrolysis	1.0 M HCl, 80°C	6	42.86
Basic hydrolysis	1.0 M NaOH, 80°C	24	75.26
Chemical oxidation	10% H_2O_2, 80°C	2	30.26

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where Ct is the concentration of buprenorphine found and Ca is the concentration of buprenorphine added to the patch.

The average recovery percentage ranged from 98.86 to 99.36%, indicating that the proposed analytical method was accurate (Table 4).

VII. *Detection Limit and Quantitation Limit*

By calculating the analytical results, the standard deviation of the Y-intercept (σ) and the slope of the calibration curve (S) were 30.54 and 12701, respectively. (see Table 5). The detection limit (DL) and quantitation limit (QL) of buprenorphine were 0.008 and 0.024 μg/mL, separately.

VIII. *Robustness*

The results for the robustness study were presented in Table 6. It showed that the HPLC performance were still acceptable by small variations of the selected factors.

IX. *Stability Studies*

The stability profiles were obtained from the results of the stability studies (see Figure 5). Both of the kinetics in Figure 5(A) and 5(B) followed the zero-order kinetics model and the rate constants were 0.13 μg/mL•hr at 60°C and 0.76 μg/mL•hr at 80°C, respectively. The reaction of buprenorphine in hydrogen peroxide followed the first-order kinetics model and the rate constant was 0.09 1/hr at 60°C as shown in Figure 5(C).

CONCLUSIONS

A rapid and effective stability indicating method has been validated according to the compendious guidelines. It can be applied to the routine analysis of buprenorphine in transdermal patch. This proposed reversed-phase HPLC method has been evaluated for the system suitability, specificity, linearity and range, precision, accuracy, detection limit, quantitation limit, robustness, and stability studies.

The advantages of this method are as follows: the peak shape is sharp and symmetrical. The retention time of buprenorphine by this method is about 7.8 minutes, comparing to 13 or 15 minutes by the recommended methods in the USP 30 or EP 5.0, respectively. The peak height of buprenorphine at 230 nm is about five times as high as that at 288 nm. Therefore, a more sensitive method can be expected. Potentially, this method can be employed in the analysis of the quality control and skin permeation of buprenorphine in the transdermal patch in the future.

Table 3. The results of the intra-day and inter-day precision

		Actual concentration (μg/mL)		
		30	50	70
Intra-day	day 1 (n=5) RSD (%)	0.08	0.22	0.15
	day 2 (n=5) RSD (%)	0.04	0.06	0.12
	day 3 (n=5) RSD (%)	0.19	0.15	0.06
Inter-day	(n=15) RSD (%)	0.88	0.65	0.77

Table 4. The results of recovery studies

Concentration of buprenorphine added (μg/mL)	Concentration of buprenorphine found (μg/mL)	RSD (%) (n = 5)	Recovery of buprenorphine (%)
30	29.83	0.44	99.25
50	49.53	0.24	98.86
70	69.69	0.30	99.36

Table 5. Data for calculating the detection limit and quantitation limit

	0.25 (μg/mL)	0.50 (μg/mL)	1.00 (μg/mL)	slope	Y-intercept
Calibration 1	3020	6018	12408	12555	175.00
Calibration 2	2999	6140	12598	12815	230.00
Calibration 3	2945	6161	12502	12734	225.50
Average	2988	6106	12503	12701	210.17
SD	38.69	77.22	95.00	—	30.54

Table 6. The robustness evaluation of the HPLC method developed

Chromatographic change factor	K' (capacity factor)	N (theoretical plate)	T (tailing factor)	Rs (resolution)
A: pH in the mobile phase				
9.3	17.99	7766	1.09	2.25
9.5	17.59	7665	1.07	2.09
9.7	17.42	7774	1.10	2.11
B: mobile phase (ACN/Buffer)				
56/44	21.17	7802	1.08	2.34
58/42	17.59	7665	1.07	2.09
60/40	14.70	7423	1.09	1.81
C: flow rate (mL/min)				
1.4	17.72	7657	1.08	2.08
1.5	17.59	7665	1.07	2.09
1.6	17.56	7652	1.08	2.09

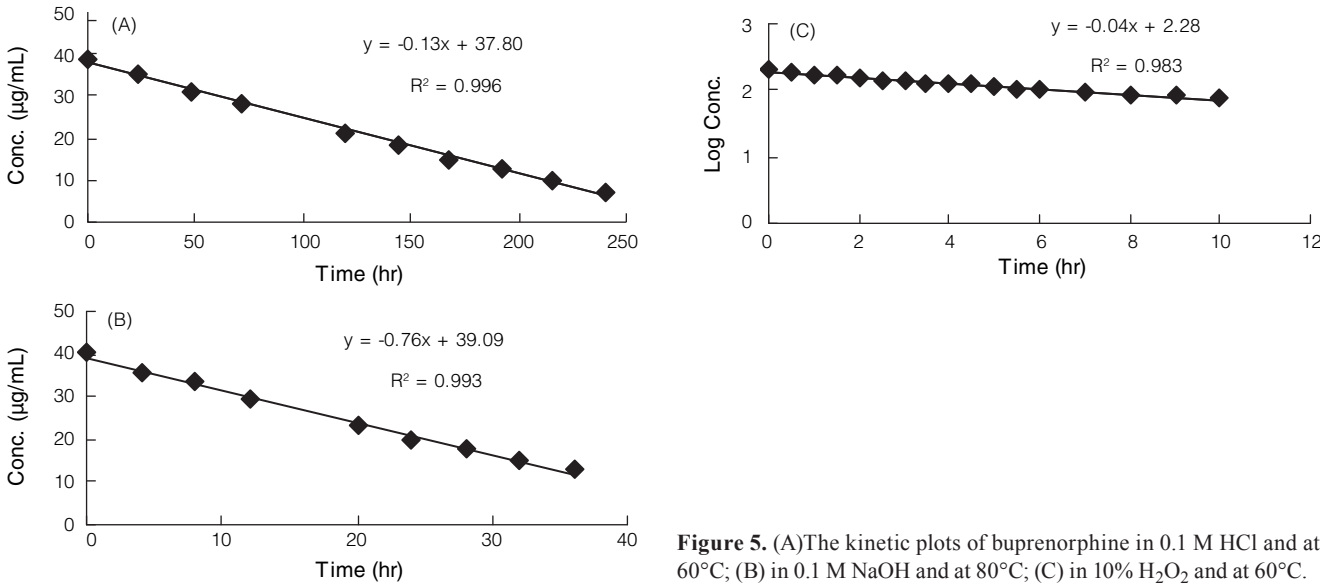

Figure 5. (A)The kinetic plots of buprenorphine in 0.1 M HCl and at 60°C; (B) in 0.1 M NaOH and at 80°C; (C) in 10% H_2O_2 and at 60°C.

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