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Original Article

SIMULTANEOUS QUANTIFICATION OF BUPRENORPHINE HCL AND NALOXONE HCL BY VIERORDT'S METHOD

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ABSTRACT

Objective: Development of a simple, rapid and accurate vierordt's method or simultaneous equation (SE) method for the simultaneous estimation of Buprenorphine HCl (BU) and Naloxone HCl (NA) in bulk and pharmaceutical dosage form. The proposed method was validated according to the guidelines of the International Conference on Harmonization and the Association of Official Analytical Chemists International.

Methods: The method was based on the measurement of absorbance at two wavelengths 289.0 nm and 283.8 nm, λ_{max} of BU and NA in methanol respectively.

Results: Calibration curves were linear in the concentration range of 40-200 µg ml^{-1} for BU and 40-260 µg ml^{-1} for NA. The mean recovery, limit of quantification (LOQ) and limit of detection (LOD) for BU were 98.91 %, 0.481 µg ml^{-1} and 0.158 µg ml^{-1} and for NA were 98.85 %, 1.283 µg ml^{-1} and 0.423 µg ml^{-1}, respectively. The method was precise, with a relative standard deviation of less than 2.0 % for both drugs. For robustness, the factors analyzed did not significantly affect the quantification of BU and NA.

Conclusion: The proposed method can be successfully applied for simultaneous estimation of BU and NA in bulk and pharmaceutical dosage form.

Keywords: Vierordt's method, Simultaneous equation method, Buprenorphine HCl, Naloxone HCl.

INTRODUCTION

Buprenorphine hydrochloride (BU) is chemically known as (6R, 7R, 14S)-17cyclopropylmethyl-7, 8-dihydro-7-[(l S)-1-hydroxy-1, 2, 2trimethylpropyl]-6-0-methyl-6, 14-ethano-17-normorphine hydro-chloride [1-4]. The molecular formula of BU is $C_{29}H_{41}NO_4$, HCl and the molecular weight is 504.1 (fig.1). It is a potent semi-synthetic opiate analgesic with a potency of 20-40 times higher than that of morphine [5]. As an analgesic, it has been used successfully by intramuscular, intravenous or sublingual routes for the treatment of moderate to severe pain as well as chronic pain [6].

Naloxone hydrochloride (NA) is chemically known as Morphinan-6-one, 4, 5-epoxy-3, 14-dihydroxy-17-(2-propenyl), hydrochloride [7, 2-4]. The empirical formula of NA is $C_{19}H_{21}NO_4$, HCl and the molecular weight is 363.84 (fig. 1). It is a potent opioid antagonist and is a competitive antagonist at mu, delta and kappa opioid receptors [6].

BU, opioid narcotic analgesic available in various dosage forms in the US market. BU has abused potential and creates dependence. Thus it is given in combination with its antagonist NA. A few examples of combinatorial dosage forms of BU and NA currently available in the market in different dosage forms by their trade names are SUBOXONE® and QUDICT® sublingual tablet; SUBOXONE® sublingual film and BUNAVAIL® buccal film.

A detailed survey of literature revealed that very few analytical methods have been reported for the estimation of BU [1, 8, 9] and NA [7, 10, 11] individually and in combined dosage form [12, 13] using High Performance Liquid Chromatography (HPLC), Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) and non-aqueous titration method. A single spectrophotometric method has been reported for estimation of BU in raw material but its estimation depended upon an ion pair formation and extraction process which is time-consuming and interference error may occur [9]. An analytical method has also been described for the estimation of BU and NA in biological fluids [14].

No spectrophotometric analytical method has yet been reported in the official compendia for the simultaneous determination of BU and NA in pharmaceutical dosage forms. The present study attempted to develop a rapid, economical, precise and accurate method for simultaneous determination of BU and NA.

The simultaneous estimation method or vierordt's method permits simultaneous analysis of both compounds without previous separation and extraction procedures. This alternative is simpler and less expensive than HPLC methods [8, 10-12].

A detailed survey of literature also revealed that scientist has previously used this simultaneous estimation method for estimation of metoprolol succinate and olmesartan medoxomil in tablet dosage form [15]; for estimation of dutasteride and tamsulosin hydrochloride in tablet dosage form by vierordt's method [16]; for estimation of paracetamol and flupirtine maleate in pure and pharmaceutical dosage form [17].

The objective of the present study was to develop a simple, precise, accurate and rapid SE method for the estimation of BU and NA in pharmaceutical dosage form.

MATERIALS AND METHODS

Apparatus

Shimadzu 1800 UV–Vis double beam spectrophotometer with UV probe software and 1 cm matched quartz cells was used.

Materials

Buprenorphine HCl USP and Naloxone HCl USP were procured from Sun Pharmaceuticals Industries Ltd. Analytical grade methanol (Merk Ltd.) was used throughout these experiments.

Standard solutions

Stock solutions of BU and NA were prepared by dissolving 20.0 mg of BU and NA separately in methanol in 50 ml volumetric flasks, and sonicated for 5 min. Final volumes of both the solutions were adjusted with methanol to get stock solutions with the concentration of 400.0 µg ml^{-1} of BU and NA.

Working standard solutions

Working standard solutions of BU was prepared by pipetting 5.0 ml of BU standard solution of 400.0 µg ml^{-1} in a 10 ml volumetric flask & working standard solutions of NA was prepared by pipetting 5.0 ml of NA standard solution of 400.0 µg ml^{-1} in a 20 ml volumetric flask. Final volumes of both the solutions were adjusted with methanol to get working standard solutions with the concentration of 200.0 µg ml^{-1} of BU and 100.0 µg ml^{-1} of NA. The concentration range of 40, 60, 80, 100, 120, 140, 160, 180, 200 µg ml^{-1} was prepared for BU and 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260 µg ml^{-1} for NA from standard solution for determination of the linearity range.

Sample solution

The sample solution was prepared using developed BU and NA sublingual tablet. A number of 20 accurately weighed tablets were ground into a fine powder using a glass mortar and pestle. A portion equivalent to 4.0 mg of BU and 4.0 mg of NA was accurately weighed and transferred to a 20 ml volumetric flask and volume was made up with methanol. The resulting solution was then sonicated for 5 min & then the final concentration of 200.0 µg ml^{-1} of BU and NA was obtained.

Method development

Selection of wavelengths

The working standard solutions of BU and NA were then scanned from 250 to 400 nm with the UV spectrophotometer using methanol as a blank. The UV spectrum of BU [18, 19] and NA [20] was shown in the fig.1; thus the λmax of BU and NA was found to be 289.0 nm and 283.8 nm in methanol, respectively & the overlain spectra of BU & NA is shown in fig. 1.

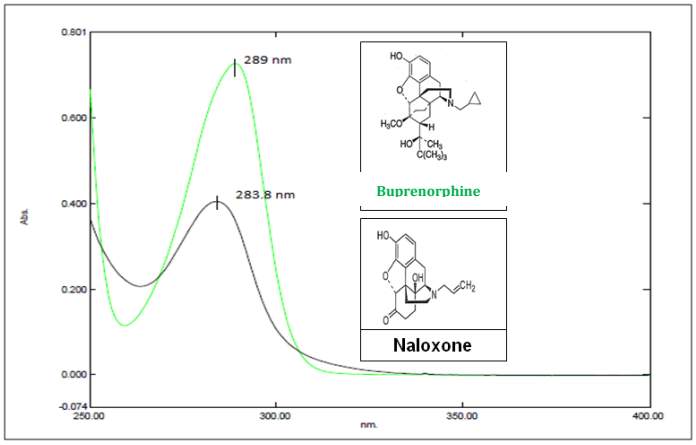

Fig. 1: Overlain spectra of buprenorphine HCl and naloxone HCl

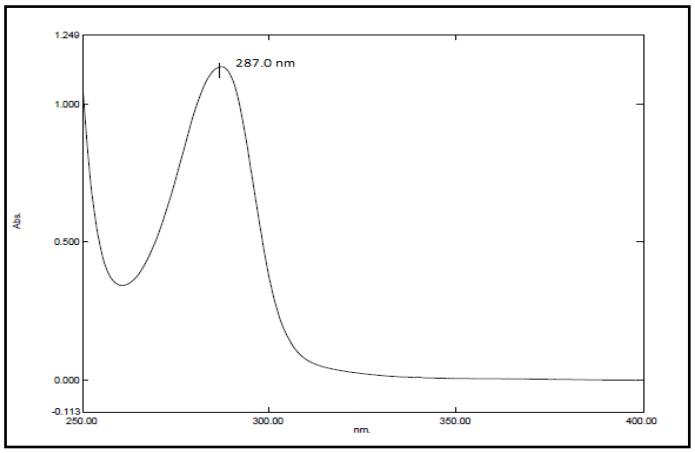

Fig. 2: UV spectra of the mixture containing buprenorphine HCl and naloxone HCl

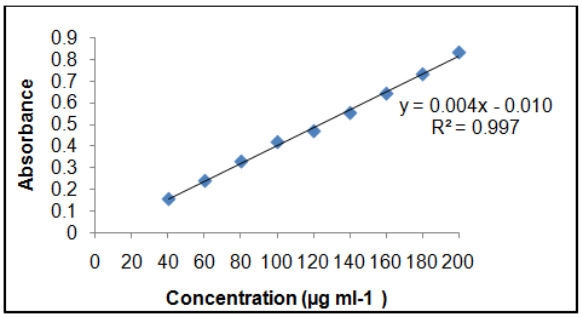

Fig. 3: Calibration graph for buprenorphine HCl

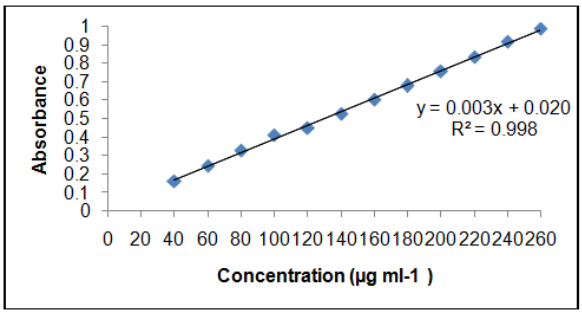

Fig. 4: Calibration graph for naloxone HCl

Validation of the concentration range

The absorbance were measured for BU and NA in the concentration range of 40, 60, 80, 100, 120, 140, 160, 180, 200 µg ml^{-1} and 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260 µg ml^{-1} at 289.0 nm and 283.8 nm for both the drugs respectively.

Calibration graphs were plotted for BU at 289.0 nm and NA at 283.8 nm at a concentration range of 40-200 µg ml^{-1} for BU and 40-260 µg ml^{-1} for NA, respectively as shown in fig. 3 & 4.

Determination of absorptivity value

The absorptivity of each solution was calculated by using the following formula:

Absorptivity = Absorbance/concentration (g/100 ml)

Theory

Simultaneous equation method OR Vierordt's method

If the sample contains two absorbing drug (X, Y) each of which absorbs at the λ_{max} of the other, then both the drugs can be quantified by using simultaneous equation method [21-23].

For solving this equation the following information is required:

(a) The absorptivities of pure drug X at λ_1 and λ_2, a_{x1} and a_{x2} respectively.

(b) The absorptivities of pure drug Y at λ_1 and λ_2, a_{y1} and a_{y2} respectively.

(c) The absorbance of diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Let c_x and c_y be the concentration of X and Y respectively in the diluted sample. Two equation are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance of X and Y.

$$\text{At } \lambda_1 \; A_1 = a_{x1} b\, c_x + a_{y1} b\, c_y \ldots\ldots\ldots \text{ (Eq. 1)}$$

$$\text{At } \lambda_2 \; A_2 = a_{x2} b\, c_x + a_{y2} b\, c_y \ldots\ldots\ldots\ldots \text{ (Eq. 2)}$$

For measurement in 1 cm cells, b=1.

The concentrations of drugs in sample solutions were determined by using the following formula:

$$C_x = A_2 a y_1 - A_1 a y_2 / a x_2 a y_1 - a x_1 a y_2 \text{ (Eq. 3)}$$

$$C_y = A_1 a x_2 - A_2 a x_1 / a x_2 a y_1 - a x_1 a y_2 \text{ (Eq. 4)}$$

Where λ_1 and λ_2 are the maximum wavelengths of BU and NA,

C_x and C_y are the concentration of BU and NA,

ax_1 and ax_2 are absorptivities of BU at 289.0 nm and 283.8 nm,

ay_1 and ay_2 are absorptivities of NA at 289.0 nm and 283.8 nm, respectively.

Method validation

Validation of this new simultaneous spectrophotometric method was carried out as recommended by the International Conference on Harmonization ICH (ICH Q2A & Q2B) guidelines [24, 25] and the Association of Official Analytical Chemists International (AOAC, 2005) [26] for the parameters of specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Specificity

The specificity of the method was evaluated through analysis of a placebo solution. The mixture of inert components was prepared in the usual concentrations employed in developed BU and NA sublingual tablet.

These solutions were analyzed by the proposed method in order to determine if any of the components of the formulation might affect the determinations of BU and NA.

Linearity

Linearity was determined from the calibration graphs plotted for BU and NA at 289.0 nm and 283.8 nm respectively. All spectrophotometric determinations were performed in triplicate and at room temperature (25±2 ˚C). The linear regression was calculated by the method of least squares, and the calibration curves were evaluated by analysis of variance (ANOVA).

Accuracy

The accuracy was calculated based on the percentage of recovery of the known amounts of BU and NA added to the samples [24-26]. Aliquots of the BU and NA standard solutions in concentrations of 200, 250 and 300 µg ml^{-1} were transferred to 10 ml volumetric flasks containing 5 ml of the sample solution. The volumes were made up with methanol and the drugs were determined in triplicate, using the proposed method.

Precision

The intra-day precision (repeatability) was evaluated by analyzing sample solutions at single concentrations of BU and NA. The analyses were performed in triplicate on the same day. To estimate the inter-day precision, the sample solutions were freshly prepared at the same concentration level for each drug, and the responses were determined in triplicate. This procedure was performed on three consecutive days. The intra-and inter-day precisions are expressed in terms of relative standard deviation (% RSD).

Limits of detection and quantitation (LOD and LOQ)

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve. The standard deviation of y-intercepts of regression lines was used as the standard deviation of responses [24-26]. These values were obtained using the following equations:

$$LOD = 3.3\ \sigma/S \text{ (Eq. 5)}$$

$$LOQ = 10.0\ \sigma/S \text{ (Eq. 6)}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

Robustness

Robustness study was carried out by changing the wavelength in ±1 nm of 289.0 nm and 283.8 nm.

RESULTS AND DISCUSSION

Method development

BU and NA are highly soluble in methanol i.e. 42.0 mg/ml and 50.0 mg/ml respectively. Hence it is used as a solvent for standard and sample preparation [27, 28]. The use of vierordt's or SE method allowed these drugs to be determined simultaneously. The absorbance values were taken at 289.0 nm for BU and at 283.8 nm for NA respectively and then the calibration curves were plotted at their respective wavelength for both the drugs as presented in the fig. 3 & 4 and their absorptivity values were calculated for each concentration as shown in table 1 & 2.

Method validation

Specificity

The specificity test demonstrated that the excipients did not affect the drug determination as depicted in the fig. 5 & 6, respectively. Thus, no interference was observed at 289.0 nm and 283.9 nm indicated that the method is specific.

Linearity

The statistical results of the linear regression for BU and NA are shown in table 3. The coefficient of determination indicated good linearity: 0.997 and 0.998 for BU and NA, respectively. The linearity ranges were 40-200 µg ml^{-1} for BU and 40-260 µg ml^{-1} for NA. The absorbance values for these concentration ranges remained between 0.1 and 0.9, conforming to the recommendations of Vogel [23]. The

RP–HPLC method for the estimation of BU & NA in pharmaceutical dosage forms shows a smaller range of concentrations [22].

Accuracy

The recovery percentages were 98.91 % and 98.85 % along with % RSD 0.639 and 0.896 for BU and NA, respectively (table 4). These results indicate the accuracy of the method.

Precision

The precision parameters (% RSD) expressed as repeatability (intraday) and as intermediate precision (inter-day) are presented in table 5. For BU and NA, all % RSD were valued were lower than 1% for repeatability in analyses performed during 3 consecutive days, and also for intermediate precision. Thus, the proposed method has good precision in the simultaneous determination of BU and NA.

Table 1: Absorptivity value for BU

Concentration (µg ml^{-1})	Absorbance λ_1 (289 nm)	Absorptivity λ_1(289 nm)	Absorbance λ_2 (283.8 nm)	Absorptivity λ_2 (283.8 nm)
40	0.155	38.75	0.143	35.75
60	0.239	39.83	0.219	36.50
80	0.328	41.00	0.302	37.75
100	0.418	41.80	0.384	38.37
120	0.471	39.25	0.432	36.00
140	0.555	39.64	0.508	36.33
160	0.643	40.21	0.590	36.85
180	0.732	40.65	0.669	37.18
200	0.834	41.72	0.769	38.45
	Mean Absorptivity at 289 nm (λ_1) (M±SD)	40.316±1.06	Mean Absorptivity at 283.8 nm (λ_2) (M±SD)	37.021±0.99

λ_1-maximum wavelength of BU, λ_2-maximum wavelength of NA, M-mean and SD-Standard deviation

Table 2: Absorptivity value for NA

Concentration (µg ml^{-1})	Absorbance λ_2 (283.8 nm)	Absorptivity λ_2 (283.8 nm)	Absorbance λ_1 (289 nm)	Absorptivity λ_1(289 nm)
40	0.161	40.25	0.142	35.50
60	0.244	40.67	0.217	36.17
80	0.330	41.21	0.294	36.79
100	0.412	41.20	0.369	36.90
120	0.452	37.67	0.404	33.69
140	0.530	37.81	0.473	33.79
160	0.605	37.83	0.542	33.85
180	0.678	37.65	0.607	33.70
200	0.758	37.87	0.678	33.90
220	0.837	38.03	0.749	34.05
240	0.916	38.14	0.820	34.18
260	0.990	38.05	0.887	34.12
	Mean Absorptivity at 283.8 nm (λ_2) (M±SD)	38.864±1.47	Mean Absorptivity at 289 nm (λ_1) (M±SD)	34.719±1.25

λ_1-maximum wavelength of BU, λ_2-maximum wavelength of NA, M-mean and SD-Standard deviation

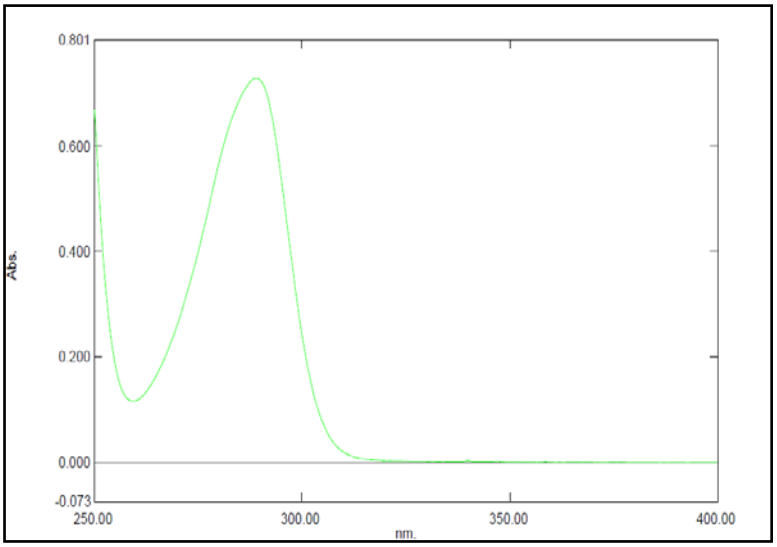

Fig. 5: Graphical evidence that no interference exists between the Buprenorphine HCl and excipients of developed sublingual tablet

Table 3: Statistical and validation parameter

Statistical parameters	BU	NA
λ_{max}	289.0 nm	283.8 nm
Linearity range (µg ml^{-1})	40-200	40-260
Regression equation (y = mx+c)	Y=0.004x-0.010	Y=0.003x+0.020
Slope (m)	0.004	0.003
Intercept (c)	0.010	0.020
Coefficient of determination (R^2)	0.997	0.998
Significant slope *(*p-value[a])	<0.0001	<0.0001
LOD (µg ml^{-1})	0.158	0.423
LOQ (µg ml^{-1})	0.481	1.283

λ_{max}–maximum wavelength, [a] Theoretical value of *p* is based on one–way ANOVA test, at α = 0.05 level of significance

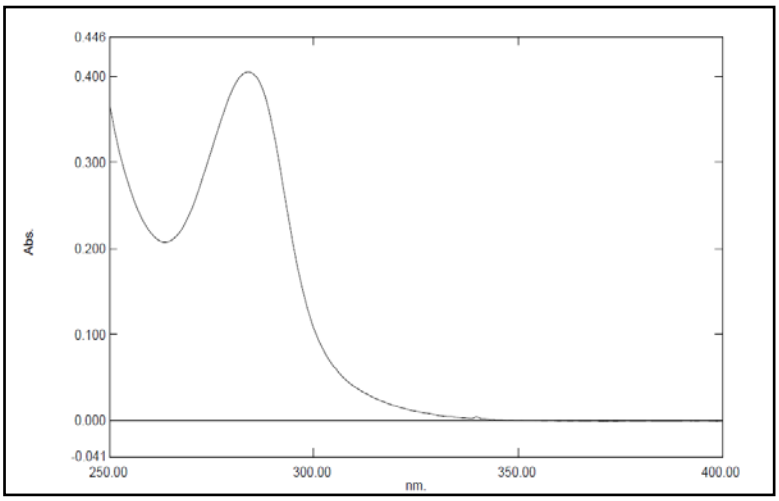

Fig. 6: Graphical evidence that no interference exists between the Naloxone HCl and excipients of developed sublingual tablet

Table 4: Recovery data for standard solutions added to the samples analysed by simultaneous equation method

Drug	Theoretical concentration (µg ml^{-1})	Experimental concentration found[a] (µg ml^{-1})	% RSD	Recovery (%)	Mean recovery (%)	% RSD
BU	120	119.69	1.092	99.74	98.91	0.639
	125	122.81	0.648	98.25		
	130	128.35	0.524	98.73		
NA	120	119.27	1.151	99.39	98.85	0.896
	125	122.56	0.619	98.04		
	130	129.37	0.666	98.52		

RSD–relative standard deviation [a]mean of 3 determinations

Table 5: Intraday and interday precision results

Parameters	Sampling time	BU					NA				
		Amount present (mg)	Amount present (%)	Mean Amount present (%)	SD	RSD (%)	Amount present (mg)	Amount present (%)	Mean Amount present (%)	SD	RSD (%)
Intraday precision	0 hr	0.200	100.291		0.072	0.072	0.265	102.136		0.074	0.072
	4 hr	0.197	98.708	99.42			0.266	102.393	102.31		
	8 hr	0.199	99.916				0.265	102.264			
Inter day precision	Ist day	0.199	99.421		1.762	1.761	0.266	102.256		0.092	0.089
	IInd day	0.200	100.083	100.01			0.266	102.649	102.77		
	IIIrd day	0.195	97.916				0.267	103.034			

SD–standard deviation and RSD–relative standard deviation

Limits of detection and quantitation (LOD and LOQ)

The LODs were 0.158 µg ml^{-1} and 0.423 µg ml^{-1} for BU and NA, and the LOQs were 0.481 µg ml^{-1} and 1.283 µg ml^{-1} for BU and NA, respectively. These values show that the proposed method has good sensitivity and results are presented in table 3.

Robustness

The responses of BU and NA did not change significantly when the analytical conditions were modified (table 6). These observations confirm the robustness of the method for determination of BU and NA in pure and pharmaceutical dosage form.

Table 6: Results obtained by changing the wavelength±1 nm

Wavelength (nm)	BU			Wavelength (nm)	NA		
	Amount present (mg)	Amount present (%)	RSD (%)		Amount present (mg)	Amount present (%)	RSD (%)
288	0.196	98.33	0.086	282.8	0.264	101.62	0.072
290	0.196	98.08	0.087	284.8	0.264	101.88	0.072

RSD–relative standard deviation

The objective of this study was to develop and validate a simple & specific UV spectrophotometric method for simultaneous determination of BU and NA. As the λ_{max} of BU and NA are closer together and not even exhibiting a difference of ±10 nm, henceforth no isoabsorption point (λ_{iso}) was exhibited as seen in fig. 2, hence estimation of both BU & NA was not possible using absorptive ratio method. Henceforth simultaneous estimation or vierordt's method was selected for determination of BU and NA in combined dosage forms. This method exhibited precise, accurate and cost effective assay for these drugs in the mixture. Based on previous studies, Mostafavi A *et al.*, (2009) developed and validated a simple isocratic RP-HPLC method for the simultaneous determination of BU, NA dehydrate and its major impurity noroxymorphone in pharmaceutical tablets [12]. The developed method was linear in the range of 0.22–220 µg mL^{-1} and 0.1–100 µg mL^{-1} for BU and NA, respectively & the recovery studies for all these three compounds were above 96%. Damodar K *et al.*, (2011) developed and validated a simple & accurate RP-HPLC method for the estimation of BU and NA in pharmaceutical dosage forms.

The developed method was linear in a range of 2.0-12.0 µg mL^{-1} for both the drugs & the RSD values for accuracy & precision studies were less than 2 % [13]. Sun W *et al.*, (2014) developed a method for the simultaneous determination of NA, BU and norbuprenorphine in human plasma using hollow fiber liquid phase microextraction combined with ultra HPLC & tandem mass spectrometry. The method was linear in the range of 0.1–25 ng mL^{-1} & the recoveries were in the range of 92.1–106.0% with RSD values were less than 15% [14]. Literature survey revealed a single spectrophotometric method for the determination of BU in raw material or in pharmaceutical formulations [9]. However, this method involved an ion pair complex formation between BU & bromocresol green which resulted into a tedious & more time-consuming process. Though above-mentioned processes for the simultaneous analysis of BU and NA are more sensitive than present method, however all the previous methodologies involved the use of highly sensitive apparatus like HPLC & hyphenated instruments such as UHPLC-MS/MS along with costly organic solvents required for mobile phase development as compared to UV spectrophotometer as employed in this study. The present method using UV spectrophotometer is more cost efficient & less time consuming.

CONCLUSION

A UV spectrophotometric method was developed and validated for the simultaneous determination of BU and NA in bulk and pharmaceutical dosage formulations. The method showed precision, accuracy, LOD, LOQ and robustness, as evaluated according to ICH guidelines. The proposed method proved to be simpler, less expensive, and faster, because no additional pre-treatment of the samples is required prior to the measuring step, thus accelerating the quality-control process. Thus the UV simultaneous equation method was suitable, useful, and an excellent alternative to HPLC to assess quality in routine analysis of BU and NA in drug products.

PERMISSION

The license was granted to M/s Zim Laboratories Ltd. by food & Drug Administration M. S. for working with Narcotic Drugs & Psychotropic Substances.

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ABBREVIATION

SE-Simultaneous equation method, BU-Buprenorphine HCl, NA-Naloxone HCl, LOQ-Limit of Quantification, LOD-Limit of Detection, HPLC-High Performance Liquid Chromatography, RP-HPLC-Reversed Phase High-Performance Liquid Chromatographic, UV-Ultra Violet

CONFLICT OF INTERESTS

All authors have none to declare

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