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RESEARCH ARTICLE

A STABILITY INDICATING ISOCRATIC REVERSED PHASE HPLC-UV METHOD FOR ASSAY DETERMINATION OF LEVO BUPIVACAINE BASE

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ABSTRACT

A isocratic reversed phase high performance liquid chromatography method was developed for the assay of levo bupivacaine base in bulk and dosage forms. The HPLC method was validated as per ICH guidelines. The chromatographic separation was achieved on a Hypersil BDS C18, 250×4.6mm,5µm column, mobile phase mix buffer using analytical grade K2HPO4, K2HPO4, KOH and acetonitrile in 1000 ml, isocratic flow rate 1.0 mL/min and system detection is performed at 235 nm has been used for HPLC-UV. Levo bupivacaine base in synthesis process and formulation. The method determination limit of specification of are the correlation coefficient was 0.999, accuracy, precision and the limits of detection and quantification found to be linear in the range of 1 µg/mL to with 300 µg/mL, recovery of assay levo bupivacaine base (99%-101%) also analyzed on newly developed HPLC method.

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INTRODUCTION

LA is an unpredictable nonattendance of pain sensation and loss of muscle power to very good decrease feeling pain in a specific area. LA is apply by injection to inject in to pain the area. LA is used two type like short and long acting for procedures. LA are anesthetics belongs two classes amino amide and amino ester. Here we select amino amide like classes of procaine to L(-) Bupivacaine.: ((S)-1-Butyl-N-(2,6-dimethylphenyl) piperidine-2-carboxamide). (Figure).L(-)BUP is a local Anesthesia (http://www.drugs.com/mtm/bupivacaine.html) drug belonging to the amino amide group, white crystalline powder that is freely soluble in 95 % ethanol, methanol, soluble in water, and slightly soluble in chloroform or acetone which has various uses (Wulfts *et al*., 1993; Markham and Faulds, 1996; Valenzuela *et al*., 1995; Najafianaraki *et al*., 2012; Abouleish *et al*., 1987; Atalay *et al*., 2010; Chestnut *et al*., 1988; Kandula and Mahesh, 2015). L(-)BUP often is administered by epidural injection total hip arthroplasty (Najafianaraki *et al*., 2012; Abouleish *et al*., 1987; Atalay *et al*., 2010; Chestnut *et al*., 1988; Kandula and Mahesh, 2015). L(-)BUP synthesis (Harold, 2011) and identification the structure is shown in (Figure 1). There are few and variety reported methods for the determination of bupivacaine hydrochloride using TLC, Gas chromatography and high performances liquid chromatography, Ultra performances liquid chromatography (Ruzafa *et al*., 1991; Huy-Riem *et al*., 1984; Morelle *et al*., 1993; Tucker *et al*., 1970; Zylber-Katz *et al*.,1978; Lindberg and Pihlajamaki, 1984; Prathyusha *et al*., 2012; Koehler *et al*., 2005; Wael *et al*., 2013), But L(-)BUP base very difficult determination on HPLC. However, these methods L(-)BUP disadvantages like the sensitivity and this work will concentrate on developing a new HPLC-UV method that is selective, simple, rapid and reproductive rapid, simple, selective and reproducible to overcome the disadvantages found in the previous methods as per procedure followed the ICH guidelines Q2A and Q2B (International Conference on Harmonization (ICH), 1996). Now a day HPLC-UV is well known, easy and widely used analytical technique for the analysis of bulk drug substance and pharmaceutical products.

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MATERIALS AND METHODS

Instrumentation and software

The HPLC system of Agilent HPLC 1100 series with variable wavelength detector (VWD). The diode array detector (DAD) microprocessor, quaternary pump, Agilent technologies international sarl,1100 series, auto sample, micro auto sample,

preparative auto sample, Thermostatic column compartment, used for this entire study. Chromatographic separation and determination was achieved on Hypersil BDS C18 250×4.6mm,5µm column, UV chamber, Analytical balance, digital pH meter, 0.45µ membrane filters.

Chemicals and reagents

The pharmaceutical API and process intermediates of L(-)BUP (99.9% pure) 5000mg were purchased from market. HPLC grade acetonitrile SD fine limited. Analytical grade K2HPO4, K2HPO4, KOH, hydrochloric acid, sodium hydroxide flakes, hydrogen peroxide. Milli-Q Water purchased from market.

Details of method chromatographic conditions

The chromatographic quantitative analysis was performed on a Hypersil BDS C18 250×4.6mm,5µm column, Mobile phase mix buffer(dissolved 2.1gm of KH2PO4 and 2.6 gm K2HPO4 in 1000ml milli-Q water filter it through 0.45µm membrane filter) and acetonitrile (30:70v/v) in 1000 ml and adjust pH 8.0 with diluted 10%v/v KOH solution, Isocratic flow rate 1.0 mL/min, Injection volume 20µL, mobile phase for diluent and system detection is performed at 235nm was used for HPLC analysis.

Preparation of solutions: Standard stock solution

Accurately weighted 50 mg of reference sample L(-)BUP and transferred into a 100 mL volumetric flask, add about 50 mL of diluent and sonication to dissolve the content. Make the volume up to the mark with diluent and mix. Conc. of L(-) BUP(500µg/mL, 500ppm).

Test solution

Accurately weighted 50 mg of test sample L(-)BUP and transferred into a 100 mL volumetric flask, add about 50 mL of diluent and sonication to dissolve the content. Make the volume up to the mark with diluent and mix. Conc. of L(-)BUP(500µg/mL, 500ppm).

Method Validation

Validation of the developed method for the determination of L(-)BUP method was performed according to the ICH guidelines "Validation of analytical procedures: text and Methodology Q2B(R),Q2B(R)" (International Conference on Harmonization (ICH), 1996) with standards bulk drug thus system suitability along with method selectivity, specificity, linearity, range, precision (repeatability) and intermediate precision, accuracy, limits of detection and quantification. L(-)BUP is short team and long term stability of the analysts in the prepared in the prepared solutions and robustness were demonstrated.

Validation Tests Study

Linearity and calibration curve limit of detection and limit of quantification

Standards for linearity at five calibration curves consisting of the validation was performed on three separate days, with seven standard calibration level lines on each day. Standard calibration curve, 10 replicates of quality control samples (deferent limit of specification)

The limit of quantification (LOQ) and limit of detection (LOD) were estimated using the following equations.

LOQ=10 б /s and LOD=3.3 б /s

Where б is the standard deviation of intercept and s is the slope of the calibration curve

Standard stock solution for linearity :

Accurately weighted 50 mg of reference sample L(-)BUP and transferred into a 100 mL volumetric flask, add about 50 mL of diluent and sonication to dissolve the content. Make the volume up to the mark with diluent and mix. Conc. of L(-) BUP(500µg/mL, 500ppm).

Precision, Accuracy, Robustness, Stability of analytical solution study

Method validation includes Precision, Accuracy, Robustness, Stability of analytical solution all study parameter have been performed on HPLC instrument as per ICH guideline Q2A (R1) and Q2B(R1) (International Conference on Harmonization (ICH), 1996).

The relative standard deviation (RSD) were estimated using the following equations. RSD =(100× SD)/AVG

where AVG is the number of results in AVERAGE result, SD is the Standard Deviation.

Calculation formula

Calculate the % Assay w/w (on an anhydrous basis) of L(-)BUP by following formula:

$$\%\ \text{Assay w/w (on an anhydrous basis)} = \frac{At}{As} \times \frac{Ws}{Wt} \times \frac{100}{100-\%\,\text{Water}} \times P$$

Where,
At=Mean peak area of L(-)BUP in test sample solution.
As=Mean peak area of L(-)BUP in standard sample solution.
Ws=Weight of L(-)BUP standard in standard sample in mg
Wt= Weight of L(-)BUP test sample in mg.
P=% Potency of L(-)BUP standard.
% Water = Water content of test sample L(-)BUP.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions for HPLC-UV assay of L(-)BUP in synthesis process and formulation. The development of HPLC methods for determining drugs has received great attention in analytical research due to its use in quality control. Choice of detection wavelength, L(-)BUP area peak was monitored at different wavelengths 210, 225, 235, 245, 250, 260 nm and a comparison wavelength and decided 235 nm on area peak was very symmetry of L(-) BUP, while baseline and minimum signal of peak (Figure 2).

Table 1. System suitability data for Assay method of L(-)BUP

	L(-)BUP		L(-)BUP
Replicate	Standard Area	Replicate	Test Area
1	10664361	1	10826753
2	10783526	2	10768526
3	10693135	3	10859135
4	10852332	4	10869732
5	10735638	5	10584596
Mean	10745798.4	Mean	10781748
Stdv	74634.94	Stdv	117046.05
% RSD	0.69	% RSD	1.09
Standard weight (mg)			
20			
Test weight (mg)			
20.00			
Label claim (mg)			
20			
Mean test weight (mg)			
20.00			
Potency			
99.95			
% Assay			
100.28			

Table 2. Summary of system suitability test with Linearity, t_R(retention time), Coefficient of determination (R^2), Y-intercept, and LOQ data for proposed method validation (% Assay) of L(-)BUP

Substance	Range (µg/mL)	t_R,(retention time)	R t_R, (relative time)	Coefficient of determination (R^2)	Y-intercept	LOQ	LOD	SLOPE
L(-) BUP	1 to 300	7.3 to 8.0	1	0.999	10776.×+142.410794.×-	8.16	2.69	10775.7
L(-) BUP	100 to 300	7.3 to 8.0	1	0.999	1929			10794

Table 3. Summary of intermediate precision and method precision comparison study data

	Set No.	L(-)BUP
Method Precision study	1	100.15
	2	100.09
	3	99.74
	4	100.01
	5	100.14
	6	100.04
	Mean	100.03
	Stdev.	0.15
	% RSD	0.15
	95% confidence level	0.12
Intermediate Precision study	1	100.29
	2	100.63
	3	100.59
	4	100.72
	5	99.93
	6	100.01
	Mean	100.36
	Stdev.	0.34
	% RSD	0.34
	95% confidence level	0.27

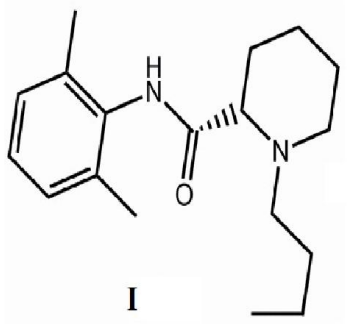

Figure 1. (I) Structure of L(-)BUP IUPAC name (S)-1-Butyl-N-(2,6-dimethylphenyl) piperidine-2-carboxamide

Table 4. Summary of accuracy, recovery, robustness and stability test study data for proposed method validation (% Assay) of L(-) BUP

Parameter	Condition	Data	L(-)BUP
Flow rate	0.9 mL/min	% RSD	0.11
	1.1 mL/min	% RSD	0.19
pH in Mobile Phase	pH (7.9)	% RSD	0.44
	pH (8.1)	% RSD	0.81
UV	230 nm	% RSD	0.21
	240 nm	% RSD	0.38
Stability at ^{0}C	at RT	Stdev.	%RSD
	Initial	68771.87	0.66
	12 hrs	80143.74	0.77
	24 hrs	90428.67	0.87
	36 hrs	93140.50	0.89
	48 hrs	99520.64	0.94
	At 5^{0}C		
	Initial	54558.99	0.52
	12 hrs	65835.65	0.63
	24 hrs	80357.14	0.77
	36 hrs	83467.51	0.81
	48 hrs	90641.62	0.86
Accuracy on level	Data		
50 %	% Recovery		99.90
	% RSD		0.09
100 %	% Recovery		99.98
	% RSD		0.07
150 %	% Recovery		100.06
	% RSD		0.20

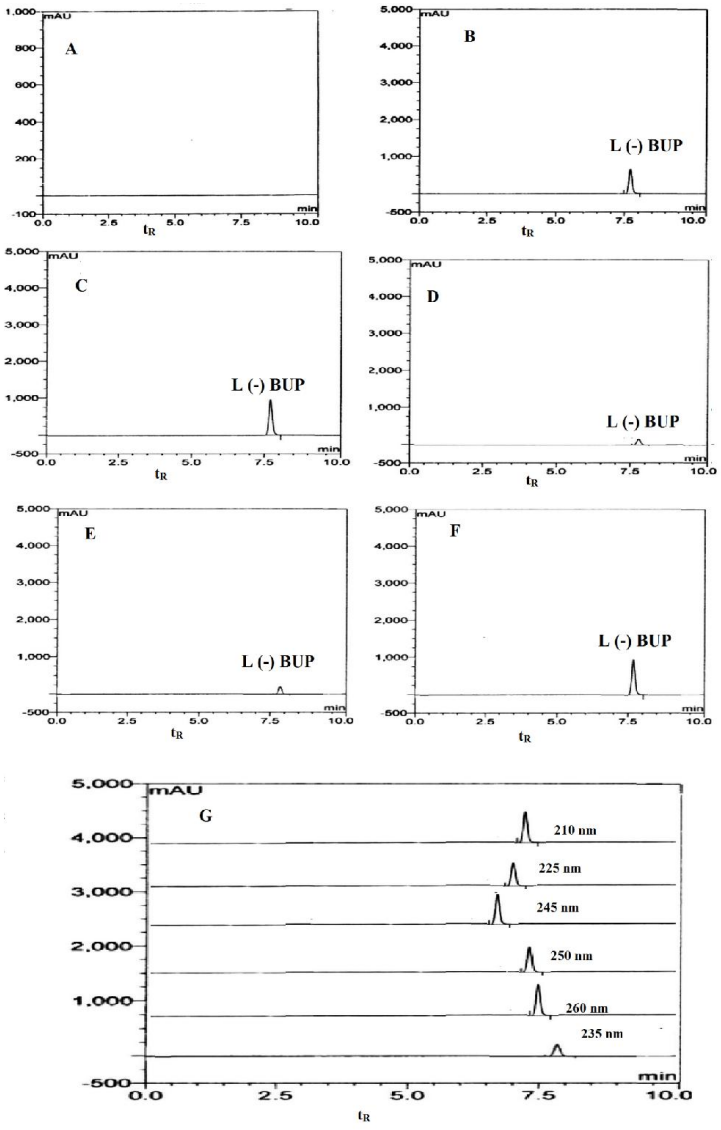

Figure 2. Chromatography of L(-)BUP (A) Blank, (B) reference of L(-)BUP solution,(C) System suitability solution,(D) LOD solution,(E)LOQ solution, (F) test solution L(-) BUP, (G) Different UV spectrum of L(-)BUP

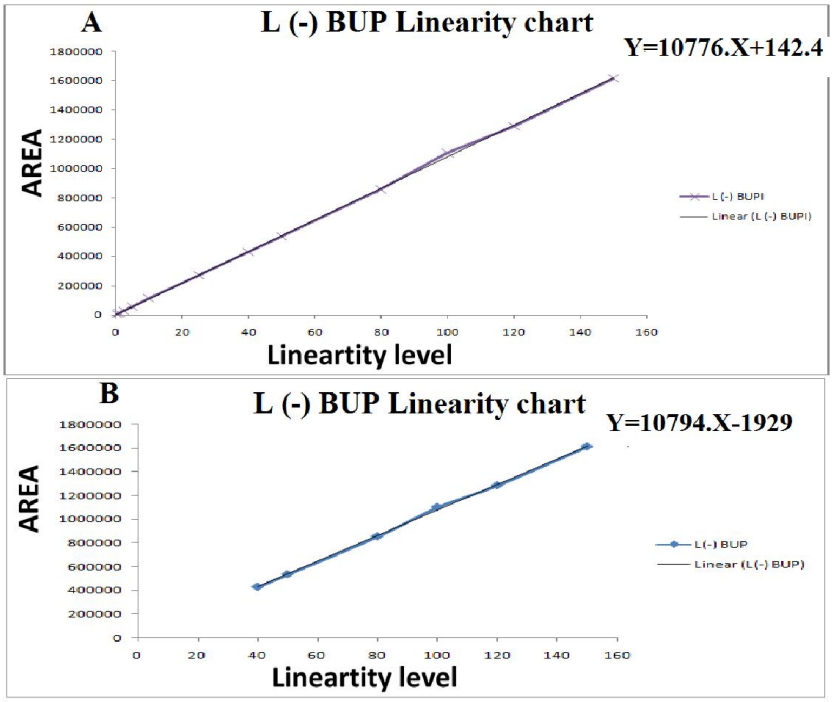

Figure 3. (A)Plot of area verses linearity level (0.5 % to 150%) and confidence level for L(-) BUP, correlation (R^2) 0.999, Y- Intercept 10776.×+142.4. (B) Plot of area verses linearity level (50% to 150%) and confidence level for L(-)BUP, correlation (R^2) 0.999, Y-Intercept 10794.×-1929. The correlation coefficient would not be less than 0.995. Y- Intercept ≤25% referred to the calculated response of the x-value corresponding to the concentration (linearity level) of the specification limit

Furthermore the UV spectra of diluents L(-)BUP showed the maximum absorption wavelength at 235 nm (Figure 2). It is easy to operate and is also well-utilized by researchers. The main objective of method development is to determine the L(-) BUP synthesis of drugs and formulation. L(-)BUP determination of Assay was achieved on a Hypersil BDS C18 250×4.6mm,5µm column, isocratic mobile phase for diluent and System detection is performed at 235nm mode solvent or equivalent column for the development of the proposed method. The method optimization of the pH of the mobile phase A and the need of and a isocratic elution. The selection of the pH of the mobile phase was very critical for pH=8.0, Finally a linear isocratic program with an initial mobile phase mix buffer(dissolved 2.1gm of KH2PO4 and 2.6gm K2HPO4 in 1000ml milli-Q water filter it through 0.45µm membrane filter) and acetonitrile (30:70v/v) in 1000 ml and adjust pH 8.0 with diluted 15%v/v KOH solution, Isocratic flow rate 1.0 mL/min, Injection volume 20µL, mobile phase for diluent and system detection is performed at 235nm was separation of the drug in synthesis process for L(-)BUP t_R (7.3-8.0).

Method validation

Method validation has been performed as per ICH guideline Q2A and Q2B includes several parameters like precision, linearity, accuracy, robustness, LOD, LOQ. These parameters all validation have been performed systematically both assay and purity on HPLC (International Conference on Harmonization (ICH), 1996). A typical chromatogram of the separation of the two analytes under these conditions is presented in (Figure 2). Results in memory Tablets are included in (Table 1), (Table 2).

Linearity and range on LC-UV

Study design: Determine the LOD and LOQ of L(-)BUP inject solution as per the following sequence. Evaluate the data and draw a linearity plot from the level, which detected to 150.0 % of specification limit. Procedure of inject blank (diluent), system suitability solution for retention time conformation, linearity level 150% (300µg/ ml) to 0.5%(1µg/ml) of specification limit. All calibration curves for L(-)BUP presented coefficient of determination (R^2) 0.999, while of determination (R^2) was greater than 0.995 as required. A lack-of-fit test was performed for all calibration curves and the calculated R^2-values of the representative curves, system suitability parameter was comply. The correlation coefficient would not be less than 0.98. Y- Intercept ≤25% referred to the calculated response of the x-value corresponding to the concentration of the specification limit. Representative linearity and range results in memory Tablets are included in (Table 1) and linearity chart show in (Figure 3).

Precision and Stability on LC -UV

The precision of the determination each of L(-)BUP was studied with respect to both repeatability and intermediate precision and method precision by one-way for six set consecutive days using the daily calibration curves five concentration levels were used for the (low, medium, high) were used and the prepared samples were analyzed in duplicate. The repeatability and intermediate precision and method precision were expressed as the % relative standard deviation (% RSD) of the analyst concentration and 95% confidence level. Comparison to between intermediate

precision and method precision included in (Table 3). At the beginning of the method development it was observed that each of L(-)BUP stock solutions prepared in diluent seemed to be very stable and no additional peaks appeared in the chromatograms. Therefore based on this observation, a stability study on L(-)BUP stock solution in diluent at RT and at 5°C for Initial,12hrs 24 hrs, 36hrs and 48hrs followed and found that they were stable and no degradation of each L(-)BUP were observed. Results for L(-)BUP are included in (Table 3), (Table 4).

Robustnesson LC-UV

The robustness of the method was evaluated by analyzing standards and test solutions at the nominal concentration of L(-)BUP (100 µg/mL). The parameters altered were the flow rate(±10%) 0.9,1.1 mL/min instead of 1.0 mL/min, pH(±0.1unit)7.9,8.1 instead of 8.0 and the different UV nm 230,240 instead 235. Representative linearity and range results in L(-)BUP drug sere included in the result were summarized in (Table 4).

Concluding Remarks

L(-)BUP was HPLC-UV Assay method was developed and validated as per ICH guidelines. The a reversed-phase performance liquid chromatography method rapid, accurate, simple and selective for the simultaneous determination L(-)BUP in the bulk drug and dosage forms. The method was fully validated and proved to be reliable sensitive, precise and robust. It is the first time that such method appears in the literature and a can be useful for routine analysis and quality control with assay limit of specification of L(-)BUP in pharmaceutical industry.

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