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SPECTROPHOTOMETRIC DETERMINATION OF BUFLOMEDIL HYDROCHLORIDE IN BULK

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ABSTRACT

Literature survey reveals that few analytical methods were reported for the estimation of Buflomedil hydrochloride by LC methods. But up to best of our knowledge there is no reported in visible spectroscopic methods for Buflomedil hydrochloride. Hence the present study aims to develope simple, rapid, precise and validated methods for buflomedil hydrochloride in bulk. The suitable solvent selected for performing estimation of Buflomedil hydrochloride by UV spectroscopic method development and validation and fixed the λ_{max} for the drug Buflomedil hydrochloride. The present study successfully estimated the Buflomedil hydrochloride from the formulation and performed validation studies of the drug Buflomedil hydrochloride.

Key words: Buflomedil hydrochloride, UV spectroscopic method, Validation studies.

INTRODUCTION

Spectroscopy is a technique that measures the interaction of molecules with electromagnetic radiation. Light in the near-ultraviolet (UV) and visible range of the electromagnetic spectrum has an energy of about 150–400 kJ mol21. The energy of the light is used to promote electrons from the ground state to an excited state. A spectrum is obtained when the absorption of light is measured as a function of its frequency or wavelength. Molecules with electrons in delocalized aromatic systems often absorb light in the near-UV (150–400 nm) or the visible (400–800 nm) region. Absorption spectroscopy is usually performed with molecules dissolved in a transparent solvent. The absorbance of a solute depends linearly on its concentration and therefore absorption spectroscopy is ideally suited for quantitative measurements. The wavelength of absorption and the strength of absorbance of a molecule depend not only on the chemical nature but also on the molecular environment of its chromophores. Absorption spectroscopy is therefore an excellent technique for following ligand-binding reactions, enzyme catalysis and conformational transitions in proteins and nucleic acids. Spectroscopic measurements are very sensitive and nondestructive, and require only small amounts of material for analysis.

Drug Profile

Buflomedil hydrochloride (Synonyms:1-[3-(2,4,6-Trimethoxybenzoyl)propyl]pyrrolidinium chloride)

Buflomedil hydrochloride is used for the treatment of Cerebrovascular and peripheral vascular disease. Dosage: PO 300-600 mg/day. IV Up to 200 mg/day via slow injection or 400 mg/day via infusion. IM Up to 100mg/day. Overdosage may lead to tachycardia, severe hypotension and convulsions. Buflomedil absorbed from the GI tract; plasma concentrations peaked at 1.5-4 hr after oral admin. Buflomedil enhances RBC deformibility, muscle cell metabolism as well as platelet inhibition, thus bringing about vasodilating effects. Elimination half-life: 2-3 hr mainly excreted in the urine as unchanged drug and metabolites.

Indication and dosage:

Oral Cerebrovascular and peripheral vascular disease Adult: 300-600 mg daily.

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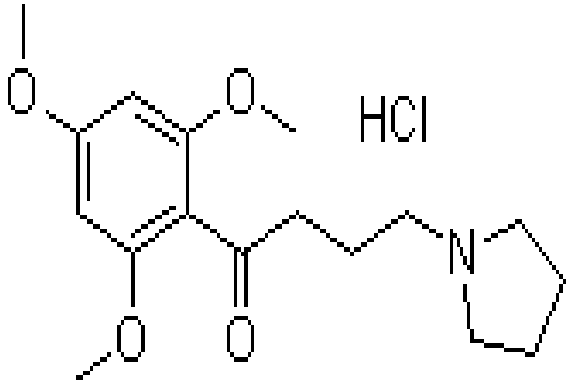
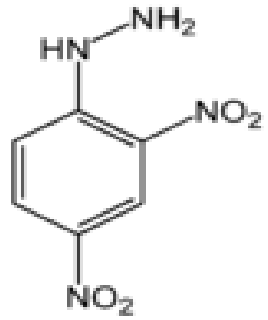
Intravenous Cerebrovascular and peripheral vascular disease
Adult: Up to 200 mg daily by slow IV Inj or up to 400 mg daily by IV infusion.
Intramuscular Cerebrovascular and peripheral vascular disease
Adult: Up to 100 mg daily.
Special Precautions: May affect ability to drive or operate machinery. Renal or hepatic Impairment.
Molecular Formula : $C_{17}H_{25}NO_4 \cdot HCl$
Molecular Weight : 343.85

Fig 1. Structure of Buflomedil hydrochloride



IUPAC name: 4-(pyrorolidin-1-yl)-1-(2,4,6-trimethoxyphenyl)butan-1-one

Reagent Profile
Fig 2. Structure of (2,4-dinitrophenyl) hydrazine



Molecular formula : $C_6H_6N_4O_4$
Molecular weight : 198.14
IUPAC name : (2,4-dinitrophenyl)hydrazine
Other names : 2,4-DNPH; 2,4-DNP; Brady's reagent
Appearance : Red or orange powder
Melting point : 198-202^{0}C
Solubility : slightly solule in water
Storge : Store in a cool, dry well-ventilated location.
Main hazards : Flammable, possible carcinogen.
Inhalation
Harmful if inhaled. Absorption into the body may cause cyanosis

Ingestion
Harmful if swallowed. May cause cyanosis.

Skin Contact
Causes irritation to skin. Symptoms include redness, itching, and pain. May cause allergic skin reactions. Absorption into body may cause cyanosis.

Eye Contact
Causes irritation, redness, and pain.

Chronic Exposure
Lengthy exposures may affect the ability of blood to carry oxygen (methemoglobinemia), resulting in bluish discoloration of lips and tongue (cyanosis).

Aggravation of Pre-existing Conditions
No information found.

MATERIALS AND METHODS

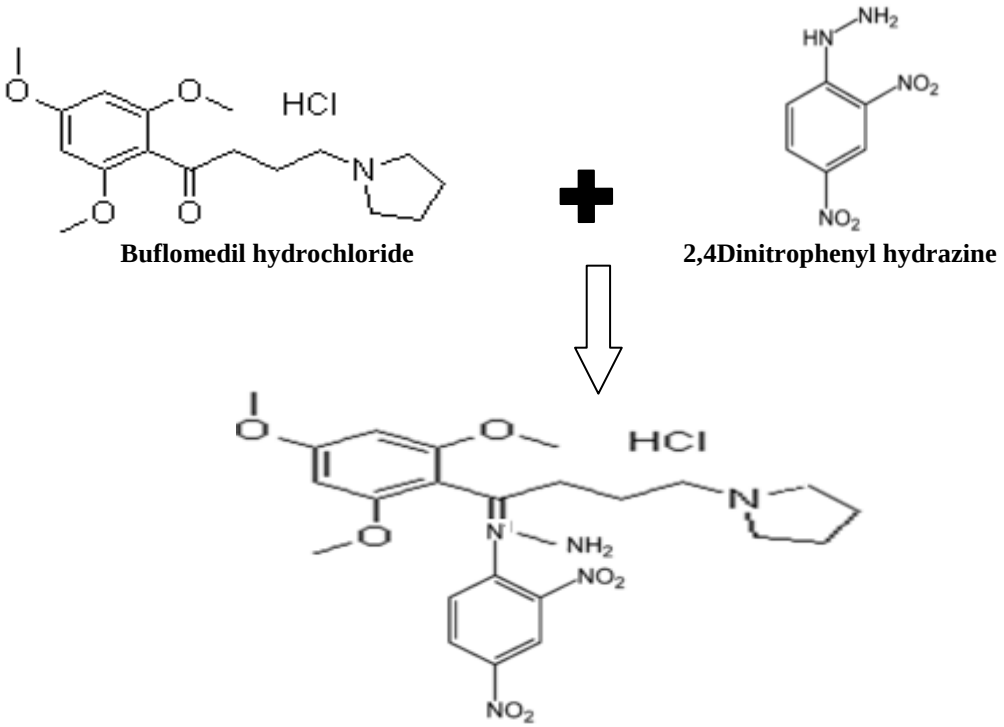
Chemicals and solvents
- Methanol AR grade,
- Ethanol
- Distilled Water
- Hydrochloric acid
- 2,4 dinitrophenyl hydrazine reagent
- Sodium hydroxide

Instruments Used
- SHIMADZU UV Pharmspec Spectrophotometer 1700
- SHIMADZU (ELB 300) Electronic balance
- SHIMADZU (BL 22OH) Electronic balance
- TOSHIBHA (India) Ultra sonicator

METHOD DEVELOPMENT OF BUFLOMEDIL HYDROCHLORIDE BY VISIBLE SPECTROSCOPIC METHOD

This method is based on the reaction of buflomedil Hydrochloride with 2,4dinitrophenyl hydrazine in presence of alkaline medium (NaOH) to form orange yellow coloured chromogen. The 2,4 dinitrochlorbenzene combines with hydrazine sulphate and forms 2,4 dinitrophenylhydrazine. Treating this complex with buflomedil Hydrochloride will give orange yellow colour. The orange yellow colour chromogen has absorbance maxima in the visible region.

Reaction



Buflomedil hydrochloride **2,4Dinitrophenyl hydrazine**

Orange yellow coloured complex

Selection of solvent for drug

Solubility of the drug in different solvents was tried. Buflomedil Hydrochloride was soluble in Methanol, Ethanol acetonitrile. As the drug showed good spectrum and was stable in Methanol, it was selected as a solvent of choice.

Selection of reagents

Primarily various reagents were treated with buflomedil hydrochloride and observed for the colour formation. The tests were carried out qualitatively using 2,4 DNP, FC reagent, $CuSO_4$ and the colour complex were observed. Blanks prepared in similar manner excluding the drug for each reagent was used to compare the colour formed by buflomedil hydrochloride. The chromogen hence formed was scanned in visible region (400-800 nm) using respective blanks. 2,4 DNP is selected as reagent as it showed a characteristic wavelength.

Selection of solvent for reagent

Reagent is soluble in ethanol in presence of conc HCl .Hence ethanol is selected as solvent of choice.

Initial colour development

The colour complex formed by buflomedil hydrochloride in acidic media (conc HCl) with NaOH and 2,4 DNP showed a characteristic spectrum and the chromogen thus formed was orangeyellow in colour. Hence NaOH and 2,4 DNP were selected for present study.

Preparation of 0.1%w/v of 2,4 Dinitrophenyl hydrazine reagent

100mg of 2,4 DNP reagent was taken and 0.5 ml of HCl and 20 ml of ethanol was added and sonicated for 5 min. Then make up the final volume to 100 ml with ethanol.

Preparation of 4% w/v NaOH

4 gm of NaOH was weighed and dissolved in distilled water and made upto 100 ml with the same distilled water.

Preparation of standard stock solution of drug

Accurately weighed about 10 mg of buflomedil hydrochloride in 100 ml standard flask and dissolved it in small amount of methanol. Then the volume was made upto 100ml with methanol (100 μg/ml).

Optimization of colorimtery parameter

To obtain an optimum experimental condition various parameters have to be optimized. They were as follows.

1. Strength of NaOH
2. Volume of 2,4 DNP
3. Volume of NaOH
4. Order of addition and stability.

Strength of sodium hydroxide solution

Different strength of sodium hydroxide in distilled water was made and tried for colour complex formation

0.04%, 2%, 4%, 8% w/v solutions were prepared and tried. The absorbances noted are shown in table 1. The colour development was minimal in 0.04-8% except 4%. Hence4 % w/v solution of NaOH was found to be more suitable.

Volume of 2,4 dinitrophenyl hydrazine reagent

Keeping the volume of drug solution and volume of sodium hydroxide, various volume of 2,4 DNP reagent was added and the study was carried out. And noted for the chromogen formation and absorbances noted are shown in table 2.

Volume of NaOH

Keeping the standard drug solution 1ml and the volume of 2,4DNP reagent as fixed 2 ml, various volume of sodium hydroxide solution was added and noted for the chromogen formation and absorbances noted shown in table 3.

Order of addition of reagent

Effect of order of addition of reagents on chromogen formation was studied by changing the order as

i) Drug +NaOH + 2,4 Dinitrophenyl hydazine reagent
ii) Drug + 2,4 Dinitrophenyl hydrazine reagent + NaOH

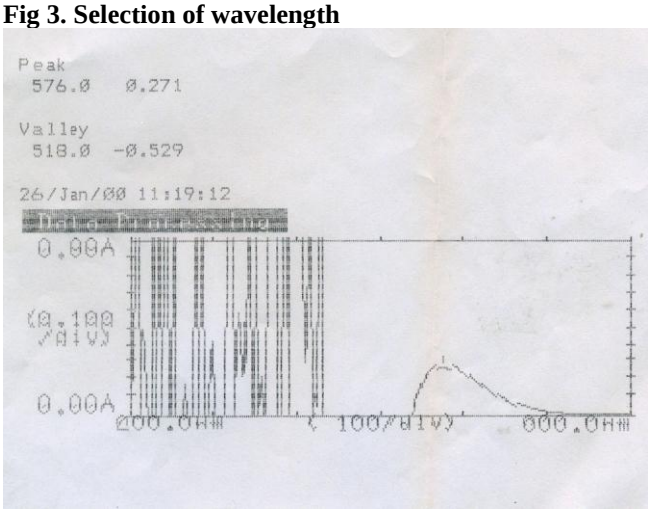
Even by changing the order of addition blue coloured chromogen was formed and λmax was576 nm. Whereas good absorption and linearity was observed only when the order is kept as Drug + 2,4 DNP + NaOH

The colour intensity of chromogen was measured at different time intervals and was found to be stable for 3 hrs whereas it was stable for only 1hr when NaOH was added followed by 2,4 DNP reagent to drug solution.

Selection of wavelength

Drug with reagent showed maximum absorbance at 576nm wavelength. Fixing 576 nm wavelength the further work was proceeded.

Fig 3. Selection of wavelength



Preparation of standard graph

Different volumes such as 2ml to 10 ml from the stock solution (100 µg/ml) were pipetted out into a series of 10 ml standard flask. To the solution 0.5ml of NaOH 4% w/v, 2ml of 2,4 Dinitrophenyl hydrazine flaks were set aside for 20 minutes. The solution were made up to the volume with distilled water: methanol in the ratio of 1:1 and mixed well. Absorbance of each solution at 576 nm were noted (table 5) and calibration graph was plotted using concentration V_S absorbance.

VALIDATION PARAMETERS

Linearity

Buflomedil Hydrochloride was found to be linear in a concentration range of 20-100 µg/ml. The absorbance of this solution was measured at 576 nm and calibration graph was plotted using absorbance Vs Concentration. The slope intercept and correlation coefficient values were found to be 0.9989 respectively.

Precision

Precision studies were performed by preparing the standards six times and measuring the absorbances of drugs at 576nm. Low %RSD shows that the method has good precision. The results are given in table 6.

Stability

Stability studies of the drug solutions were carried out and they were found to be stable at room temperature for about 3hours.

LOD (Limit of detection)

LOD is defined as the lowest concentration of an analyte that analytical process scans reliably differentiate from back-ground levels. In this study LOD and LOQ were based on the standard deviation of response and the slope of corresponding curve using following equation:

$$\mathbf{LOD = 3.3\ \sigma / S}$$

Where σ is standard deviation of Y-intercept and S is slope of calibration curve shown in the table 7.

LOQ (Limit of quantification)

LOQ is defined as the lowest concentration of calibration curve that can be measured within acceptable accuracy, precision and variability. The value of LOQ was determined using following equation:

$$\mathbf{LOQ = 10\ \sigma / S}$$

LOQ (Limit of Quantification) Value is the minimum quantity of drug that can be quantified by the instrument and shown in the table 7.

Beers Limit

The limits in which beers law obeyed is beers limit. In this method development the accuracy, precision the ruggedness, robustness is showed within range called Beers limit, the beers limit range found to be 20µg/mL to

100µg/ml. Within this range the drug shows accuracy, linearity, precision, ruggedness, robustness.

RESULTS

Buflomedil along with the reagent showed maximum absorbance 576 nm and obeyed beers law in the concentration range of 20 to 100 µg/ml.

Optimisation

Table 1. Selection of Strength of sodium hydroxide

Strength of sodium hydroxide (% w/v)	Colour	Absorbance (576 nm)
0.04	yellow colour	0.078
2	Light Yellow colour	0.354
4*	**Orange yellow colour**	**0.602**
8	Orange yellow colour	0.423

*selected parameter

Table 2. Selection of volume of reagent

Volume of 2,4 dinitrophenyl hydrazine (ml)	Absorbance (576 nm)
0.5	0.10
1	0.065
1.5*	**0.092**
2	0.106
2.5	0.07

* Selected parameter

Fig 4. linearity graph

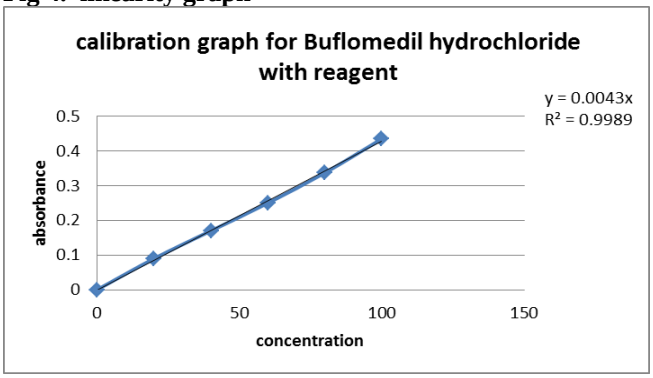


Table 3. Selection of volume of sodium hydroxide (0.1% w/v)

Volume (ml)	Colour	Absorbance (576nm)
0.5*	**Orange yellow colour**	**0.271**
1 ml	OrangeYellow colour	0.153
1.5 ml	Orange yellow colour	0.085
2ml	OrangeYellow colour	0.063

* Selected parameter

Table 4. Stability for order of addition

Time (mins)	Absorbance (576 nm)	
	(i)	(ii)
10	0.135	0.132
15	0.141	0.131
20	0.149	0.127
30	0.152	0.116
1 hr	0.147	0.108
2 hr	0.133	0.099
3 hr	0.11	0.085

Table 5. Calibration graph of Buflomedil Hydrochloride with reagent

Concentration (µg/ml)	Absorbance (576nm)
20	0.09
40	0.17
60	0.25
80	0.337
100	0.435

Table 6. Precision

Conc. (µg/ml)	Absorbance At 576nm	% RSD
20	0.110 0.109 0.107	1.57

Table 7. LOD &LOQ

contents	Wavelength at 576nm
LOD	**0.95**
LOQ	**1.28**

Table 8. Method Validation parameters

Parameters	Values
λ_{max} (nm)	576
Linearity range (µg/ml)	20-100
Colour stability (hr)	3hr
Intercept (a)	0.00033
Slope (b)	0.0043
Correlation coefficient	0.9989

- Interday and intraday precision was performed and the %RSD was found to be less than 2 and was shown in the table 7.
- LOD& LOQ are shown in the table 8.
- Stability studies were shown in the table 4.

DISCUSSION AND CONCLUSION

Buflomedil hydrochloride is a vasoactive drug. This drug is to treat claudation or symptomps of peripheral arterial disease. Buflomedil hydrochloride is indicated in the treatment of intermittent claudication, ischemic rest pain and trophic disorders or skin ulcerations due to chronic arterial insufficiency, as well as in Raynaud's disease, Raynaud's syndrome and other vasospastic disorders such as live and acrocyanosis. Buflomedil hydrochloride is further indicated in the treatment of signs and symptoms often associated with either chronic cerebrovascular insufficiency or aging: vertigo, tinnitus, dizziness, intellectual deterioration, changes in personality and socio-familial behavior, insomnia, loss of memory, especially recent memory, loss of concentration, disorientation.

By trial and error method suitable chromogenic reagent was selected. 2,4 Dinitrophenyl hydrazine proved to be a better chromogenic agent. It was found that reagent was soluble in ethanol in presence of con. HCl. Then the concentration and the volume of reagent was optimised. 2ml of reagent was used. In presence of NaOH the colour was developed and volume and strength of NaOH was optimised i.e, 0.5 ml of 4%w/v. The λmax was found at 576 nm and calibration graph was plotted and the linearity was obtained in the range of 20 to 40µg/ml. The slope, intercept and the correlation factor of Buflomedil hydrochloride at 576 nm was found to be 0.0043, 0.00033 and 0.9989 respectively. The method was validated and the precision, linearity, LOD, LOQ were calculated. Interday and intraday precision were performed at 576 nm and the %RSD was found to be 1.57 which is <2. Stability studies were performed the reagent along with the drug was stable for 3 hrs. Beer's limit was found in the concentration range of 20-10 0µg/ml. Within this range the drug shows, linearity, precision, ruggedness, robustness.

The proposed visible spectrophotometric method for Buflomedil Hydrochloride in bulk form is simple, rapid, precise and can be employed for routine analysis. This method is simple, cost effective and can be employed in laboratories. Low %RSD values indicate that the method was precise & reproducible. This method can be applied for substances obeying Beer's law.

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