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Research Article

# COMPARISON OF RP-HPLC AND UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF HALOPERIDOL IN PURE AND PHARMACEUTICAL FORMULATION

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### ABSTRACT

An accurate, precise, sensitive and reproducible High-performance liquid chromatographic (HPLC) and UV spectrophotometric methods were developed and validated for the quantitative determination of haloperidol (HPD) in bulk drug and pharmaceutical formulation. Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The RP-HPLC method was developed by the isocratic technique on a reversed-phase Thermo C18 (250 × 4.6 mm, 5µm) column with mobile phase consisting of Methanol: Acetonitrile (50:50v/v) at flow rate of 1.0 ml/min. The retention time for HPD was 2.238±0.3min. The UV spectrophotometric determinations were performed at 244 nm using 80% methanol as a solvent. The linearity range for HPD was 5-25 µg/ml for both HPLC and UV method. The linearity of the calibration curves for each analyte in the desired concentration range was good (r<sup>2</sup> >0.999) by both the HPLC and UV methods. The method showed good reproducibility and recovery with percent relative standard deviation less than 2%. Moreover, the accuracy and precision obtained with HPLC correlated well with the UV method which implied that UV spectroscopy can be a cheap, reliable and less time consuming alternative for chromatographic analysis. The proposed methods are highly sensitive, precise and accurate and hence successfully applied for determining the assay and in vitro dissolution of a marketed formulation.

**Keywords:** HPLC, UV Spectrophotometry, Haloperidol, Pharmaceutical formulation, Method validation, Quantitative analysis.

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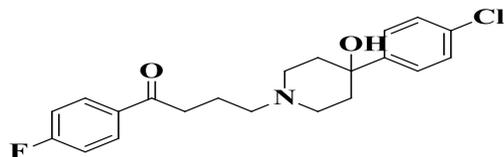
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### INTRODUCTION

Haloperidol (HPD) is a tertiary amine that occurs as a white or almost white powder, is practically insoluble in water and is slightly soluble in alcohol, methanol and methylene chloride<sup>1</sup>. Haloperidol is an orally administered dopamine inverse agonist of the typical antipsychotic class of medication that chemically belongs to butyrophenone group<sup>2</sup>. Its mechanism of action is mediated by blockade of D2 dopamine receptors in the mesocortex and the limbic system of the brain<sup>3</sup>. Haloperidol is used to treat certain psychiatric conditions including schizophrenia, maniac states, medicament induced psychosis and neurological

disorders with hyperkinesias<sup>4</sup>. It is also used to treat extreme behavior problems in children and to ease the symptoms of tourett's syndrome. The dose of haloperidol for the treatment of schizophrenia is 5-15mg/day with an average of 10mg per day. Its therapeutic plasma concentrations are in the range of 4–20ng/ml. Most common dosage forms are tablets, oral solutions and injections. Side effects related to haloperidol are extrapyramidal including acute dystonic reactions, akathisia syndrome, drug induced Parkinsonism, bradykinesia and tardive dyskinesia<sup>5</sup>. Haloperidol is chemically 4-[4-(4-chlorophenyl)-4-hydroxypiperidino] 4'- fluorobutyrophenone (Fig.1).The molecular formula of haloperidol is C<sub>21</sub>H<sub>23</sub>ClFNO<sub>2</sub> and

molecular weight is 375.86 g/mol. It is officially recognized in Indian Pharmacopoeia<sup>6</sup>, British Pharmacopoeia<sup>7</sup>. Haloperidol is metabolized to several metabolites<sup>8</sup> and the enzymes involved in the biotransformation of haloperidol include cytochrome P450, carbonyl reductase and uridine diphosphoglucose glucuronosyltransferase. The greatest proportion of the intrinsic hepatic clearance of haloperidol is by glucuronidation followed by the reduction of haloperidol to reduced haloperidol and by cytochrome P450-mediated oxidation<sup>9</sup>.



**Figure 1: Chemical structure of haloperidol**

Various analytical techniques have been used for determination of haloperidol individually and in combination with other drugs in pharmaceutical formulations, in human serum, human plasma and urine by RP- HPLC<sup>10-19</sup> and UV spectrophotometry<sup>20-23</sup>, High performance thin-layer chromatography (HPTLC)<sup>24</sup>, 19F NMR spectroscopy<sup>25</sup>, square-wave adsorptive stripping voltammetry at a mercury electrode<sup>26</sup>, Non-aqueous titrimetric method has also been developed for haloperidol determination in pharmaceutical preparations<sup>27</sup>. However there is no combine method available for the determination of HPD drugs. Therefore, an attempt was made to develop a new, rapid and sensitive method for the determination of HPD in bulk drug and pharmaceutical formulation. To assess the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines<sup>28</sup>.

## EXPERIMENTAL

### Reagents and chemicals

Pure sample of Haloperidol was received as a gift sample from Vamsi Labs. Ltd. Sholapur, Maharashtra, India. Acetonitrile (HPLC Grade), methanol (HPLC Grade), supplied by Merck Ltd, New Delhi, India. Triple distilled water was generated in house. Tablet Depidol 5mg Torrent Lab. Pvt. Ltd. Ahmedabad, India was purchased from local market.

### Instrument

In UV-spectrophotometric method, Labindia model-3000 + series were used, which is a wavelength accuracy  $\pm 1$  nm, with 1cm quartz cells.

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

### UV spectrophotometric method

#### Determination of wavelength of maximum absorbance ( $\lambda_{max}$ ) of HPD

Wavelength of maximum absorption was determined by scanning 10 $\mu$ g/ml solution of HPD using UV

spectrophotometer from 200 to 400 nm. This showed maximum absorbance at 244.0 nm (Fig. 2).

### Preparation of standard stock solution

10 mg of HPD was weighed accurately and transferred to a 10ml volumetric flask and the volume was adjusted to the mark with the diluents (Methanol: water 80:20 v/v), to give a stock solution of 1000ppm.

### Preparation of working standard solution

From stock solutions of HPD, 1 ml was taken and diluted up to 10 ml, from this solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with 80% methanol gives standard drug solution of 5, 10, 15, 20, 25 $\mu$ g/ml concentration.

### Preparation of the calibration curves of the drug

The calibration curve was prepared by scanning test samples ranging from 5-25 $\mu$ g/ml at 244 nm for HPD. The calibration curve was tested by validating it with inter-day and intra-day measurements. Mean of n =5 determinations was plotted as the standard curve (Fig.3).

### RP-HPLC method

#### Chromatographic condition

The isocratic mobile phase consisted of Methanol: Acetonitrile in the ratio of (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/min. The mobile phase was filtered through nylon 0.22  $\mu$ m membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5  $\mu$ m, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 244 nm was selected as the detection wavelength for UV-Visible detector.

### Standard preparation

#### Standard stock solution

10mg of HPD was weighed accurately and transferred to separate 10 ml volumetric flask, and the volume was adjusted to the mark with methanol to give a stock solution of 1000  $\mu$ g/ml.

#### Working standard solution

From stock solutions of HPD 1 ml was taken and diluted up to 10 ml with methanol. From this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25  $\mu$ g/ml concentration.

### Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 5-25  $\mu$ g/ml for HPD solutions manually in triplicate to the HPLC system at detection wavelength of 244 nm. Mean of n =5 determinations was plotted as the standard curve (Fig.4). The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both inter day and intra-day measurements.

### System Suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 µg/ml of HPD were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in table 1.

### Validation Parameters

#### Linearity

Linearity was studied by analyzing five standard solutions (n=5) in the range of 5-25 µg/ml of HPD in both UV spectrophotometric and HPLC method. Calibration curves with concentration versus absorbance or peak area was plotted for each method and the obtained data were subjected to regression analysis using the least squares method. Linearity of HPD was established by response ratios of drug. Response ratio of drug was calculated by dividing the absorbance or peak area with respective concentration (Table 2).

#### Accuracy

The validity and reliability of the proposed methods was assessed by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The recovery studies were carried out by adding known amount of standard solution of HPD to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. In UV Spectrophotometric method, the value of mean recoveries was found to be in the range of 99.58 % to 99.89 % for HPD. The value of SD and %RSD less than 2 indicate the accuracy of the method. In RP-HPLC method, the value of mean recoveries was found in the range of 99.88% to 100.07 % for HPD. Total amount of drug found and percentage recovery was calculated. Results of recovery studies are reported in Table 3.

#### Specificity

The method specificity was assessed by comparing the chromatograms (HPLC) and scans (UV) obtained from the drug and the most commonly used excipient mixture with those obtained from blank (excipient solution in water without drug). The excipients chosen are the ones used commonly in tablet formulation, which included dicalcium phosphate (DCP), lactose, starch, microcrystalline cellulose (MCC), polyvinyl pyrrolidone (PVP), sodium starch glycolate (SSG) and magnesium stearate. The drug to excipient ratio used was similar to that in the commercial formulations Fig 5.

#### Precision

Precision was determined by repeatability and intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 4.

### Robustness

For the robustness of the analytical method we changed the ratio of mobile phase. As a replacement for the methanol: acetonitrile in a ratio of 50:50v/v, methanol: acetonitrile in a ratio of 55:45 v/v were used as solvent (Results are shown in Table 4).

### LOD and LOQ

LOD and LOQ of described method were observed as 0.404µg/ml and 1.200µg/ml for HPD in UV spectrophotometric method and 0.570 µg/ml and 0.500 µg/ml for HPD in RP-HPLC method, based on the SD of response and slope, which meet the requirement of new method.

### Analysis of marketed formulation

20 tablets (Depidol 5 mg) were weighed and ground to a fine powder. An equivalent amount to 5 mg of HPD was taken in 10 ml volumetric flask. This was dissolve in 5 ml of diluents by sonication for about 10 minutes. The volume was made up to the mark by diluents as per the UV spectrophotometry method and RP-HPLC method. The solutions were filtered (whatman filter paper no.41). The filtrate was used to prepare samples of different concentration. The statistical evaluation of tablet analysis by both methods is reported in Table 5.

## RESULT AND DISCUSSION

RP-HPLC and UV-Spectrophotometric methods were developed for HPD which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The chromatographic conditions were optimized in order to provide a good performance of the assay. The retention times (Rt) of HPD was 2.238±0.3min. The chromatograms have been shown in Fig. 6. A five point calibration curve was constructed with working standards and was found linear (r<sup>2</sup> = 0.999) for each of the analytes over their calibration ranges. The slopes were calculated using the plot of drug concentration versus area of the chromatogram. The developed HPLC method was accurate, precise, reproducible and very sensitive.

For UV Method:  $Y = 0.049x + 0.006$  (r<sup>2</sup>= 0.999)

For RP-HPLC:  $Y = 120.7x + 31.48$  (r<sup>2</sup>= 0.999)

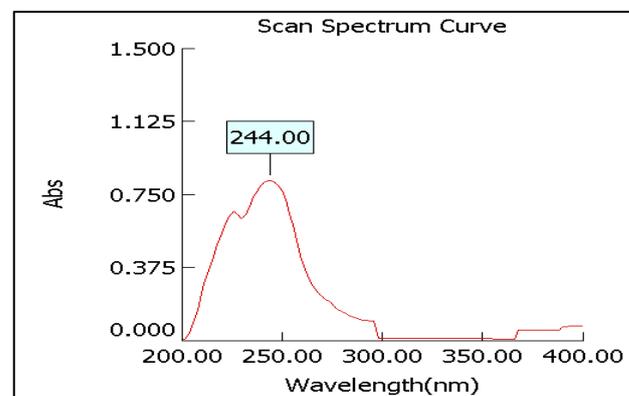


Figure 2: Selection of  $\lambda_{\text{max}}$  of haloperidol

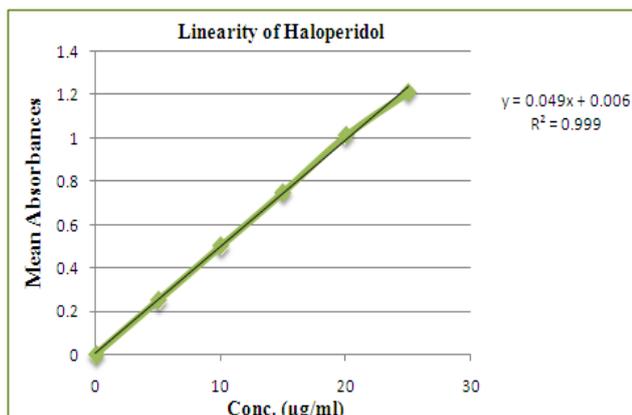


Figure 3: Calibration curves of the haloperidol

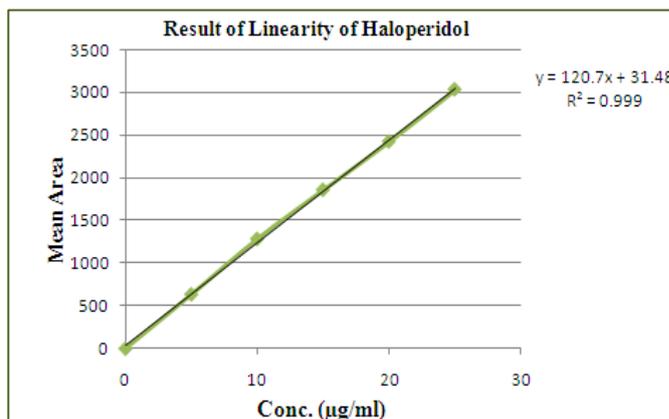


Figure 4: Calibration graph of haloperidol

Table 1: Results of system suitability parameters

Parameters	Haloperidol
AUC	1285.245
No. of Theoretical Plates	3039.000
Tailing Factor	1.173
Retention time	2.299

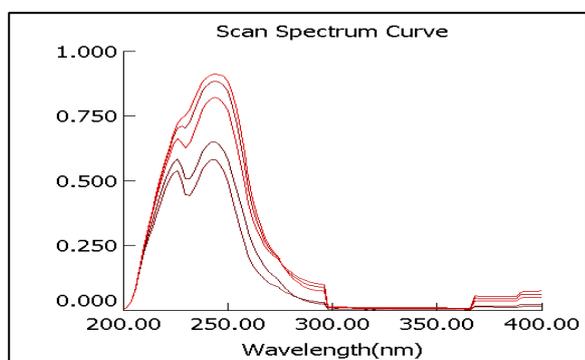
Table 2: Response ratios of HPD

Concentration (µg/ml)	HPLC Method		UV Method	
	AUC	RR	ABS	RR
Haloperidol				
5	635.654	127.131	0.250	0.050
10	1290.236	129.024	0.501	0.050
15	1874.458	124.964	0.751	0.050
20	2436.654	121.833	1.012	0.050
25	3045.589	121.824	1.214	0.048

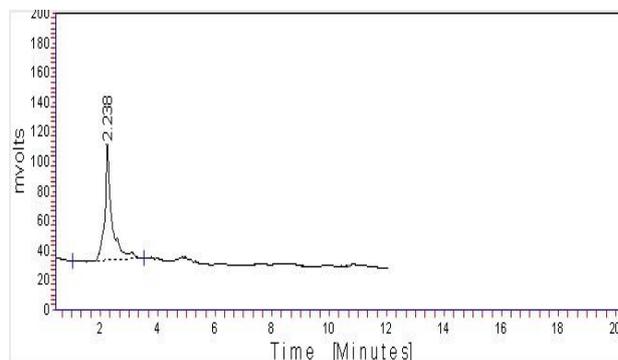
Table 3: Results of recovery study

Recovery Level%	% Mean±SD*	
	U.V Method	RP-HPLC Method
80%	99.58±0.629	100.07±0.994
100%	99.93±0.416	100.03±0.208
120%	99.89±0.834	99.88±0.278

\* Value of three replicate and three concentrations



(A)



(B)

Figure 5: UV scans (A) HPLC chromatograms (B)

**Table 4: Results of precision**

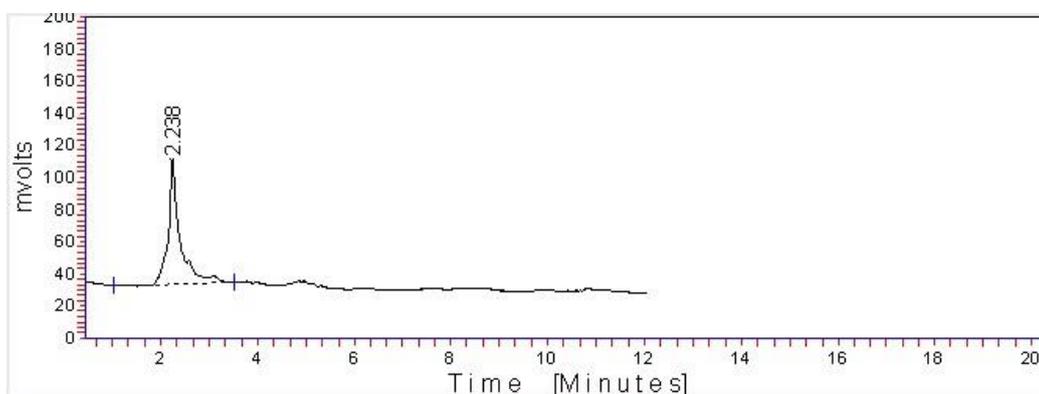
Parameters	UV Method		RP-HPLC Method	
	HPD	%RSD	HPD	%RSD
Precision (Mean± SD)*				
Repeatability	98.30±0.071	0.071	99.42±0.160	0.160
Day to Day	99.57 ±0.109	0.109	99.13 ±0.546	0.550
Analyst to Analyst	99.00±0.011	0.011	99.60 ±0.110	0.110
Robustness*	-	-	99.24 ±0.170	0.171

\*Average of 5 replicate and 5 concentration.

**Table 5: Results and statistical parameters for tablet analysis**

S. No	Drug	Label claim	Amount found	% Label claimed	SD*	%RSD*
UV Method	HPD	5	4.98	99.60	1.61	1.61
RP-HPLC	HPD	5	4.95	99.00	1.83	1.84

\*Average of five determination

**Figure 6: Chromatogram of HPD**

All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines. Table 3 lists the percent recovery (content uniformity) of HPD in the commercial formulations by HPLC and UV methods. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 4). Moreover the %R.S.D. (less variation) shows good precision of both developed methods. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. The methods were specific as none of the excipients interfered with the analytes of interest. Hence, the methods were suitably employed for assaying HPD in commercial marketed formulation (Table 5).

## CONCLUSION

The advantage of UV method over HPLC method is that the proposed UV method does not require the elaborate treatment and procedures usually associated with chromatographic method. It is less time consuming and economical. A statistical comparison of the quantitative determination of HPD shows that HPLC method as more accurate and precise than UV method. The results indicate HPLC and UV spectrometry methods are adequate methods to quantify HPD in pure form and its dosage form. There was no interference by excipients in the tablets and the mobile phase is easy to prepare. Since these methods are simple, specific, rapid and accurate, they may be successfully and conveniently adopted for routine quality control analysis of HPD in bulk and pharmaceutical dosage form.

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