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Development and validation of a stability indicating UPLC method for simultaneous determination of haloperidol and benzhexol in pharmaceutical combined dosage forms

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Abstract
A rapid, accurate, and precise UPLC method was developed for the simultaneous estimation of Haloperidol (HPD) and Benzhexol (BZH) in bulk and combined dosage forms. Chromatographic separation was achieved on a BDS C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of acetonitrile and 0.1% orthophosphoric acid (55:45, v/v) at a flow rate of 1 mL/min, with detection at 210 nm. The method was validated as per ICH guidelines, exhibiting excellent linearity, accuracy, precision, and robustness. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed the method's stability-indicating capability, with no interference observed from degradation products. The developed method is simple, reliable, and well-suited for routine quality control and stability assessment of Haloperidol and Benzhexol in pharmaceutical formulations.

Keywords: UPLC, haloperidol, benzhexol, stability-indicating, method validation

Introduction
The simultaneous determination of multiple active pharmaceutical ingredients (APIs) in combined dosage forms has become increasingly critical for quality control and regulatory compliance. Haloperidol, a typical antipsychotic, is widely prescribed for the management of schizophrenia, acute psychosis, and Tourette syndrome due to its dopamine D_2 receptor antagonism, providing effective control of psychotic symptoms [1]. Benzhexol, also known as trihexyphenidyl, is an anticholinergic agent commonly used to alleviate extrapyramidal side effects associated with haloperidol therapy, improving patient tolerability and compliance [2, 3].

Analytical methods capable of accurately quantifying both compounds simultaneously are essential to ensure dosage uniformity, stability, and therapeutic efficacy. High-performance liquid chromatography (HPLC) and its advanced form, ultra-performance liquid chromatography (UPLC), have been widely employed for this purpose due to their high resolution, sensitivity, and rapid analysis time [4, 5]. UPLC, in particular, allows for reduced solvent consumption and shorter run times while maintaining superior separation efficiency, making it highly suitable for routine quality control in pharmaceutical laboratories [6].

Despite existing methods for individual determination of haloperidol and benzhexol, there is a need for a validated, stability-indicating UPLC method for their simultaneous estimation in combined formulations. Such a method must be robust, precise, accurate, and capable of detecting potential degradation products under stress conditions, following ICH Q2(R1) guidelines for analytical method validation [7].

This study aims to develop and validate a rapid, sensitive, and reliable UPLC method for the simultaneous estimation of haloperidol and benzhexol, including forced degradation studies to ensure its stability-indicating capability.

Materials and Methods
Chemicals and Reagents
Haloperidol (HPD) and Benzhexol (BZH) reference standards were obtained from Spectrum

Pharma Research Solutions, Hyderabad, India. HPLC-grade solvents including acetonitrile and methanol were procured from Ranchem Private Limited. Analytical-grade orthophosphoric acid and perchloric acid were used for buffer preparation. Ultra-pure water was produced using an in-house Milli-Q water purification system. All chemicals and reagents complied with analytical standards [1, 2].

Instruments and Software

Chromatographic analysis was performed using a Waters UPLC system (Alliance 2695) equipped with a quaternary pump, autosampler, and 2996 PDA detector, controlled by Empower 2 software. Separation was achieved on a BDS C18 column (250 × 4.6 mm, 5 µm). Additional instruments included a digital pH meter (Elico), an ultrasonicator (PCI Analytics), and an analytical semi-micro balance (Sartorius). Membrane filtration was performed using 0.45 µm nylon filters (Pall Life Sciences).

Chromatographic Conditions

The optimized method employed a C18 reverse-phase column with an isocratic mobile phase consisting of 0.1% orthophosphoric acid buffer and acetonitrile in a 45:55 (v/v) ratio. The flow rate was maintained at 0.3 mL/min, and the injection volume was 2 µL. Detection was carried out at 210 nm, corresponding to the λmax of both drugs. The column temperature was maintained at 30°C, and the run time was 4 minutes. Water: acetonitrile (50:50 v/v) was used as a diluent for both standards and samples [3, 4].

Preparation of Standard Stock Solutions

Haloperidol Standard: Accurately weigh 10 mg of haloperidol and dissolve in 10 mL of diluent to obtain 1000 µg/mL stock solution.

Benzhexol Standard: Accurately weigh 2 mg of benzhexol and dissolve in 10 mL of diluent to obtain 200 µg/mL stock solution. Working standards were prepared by serial dilutions to cover the linearity range (HPD 50-300 µg/mL; BZH 10-60 µg/mL) [5].

Preparation of Sample Solution

Tablet powder equivalent to one dosage unit was accurately weighed and transferred to a 10 mL volumetric flask. The diluent was added, and the mixture was sonicated for 15 minutes to ensure complete dissolution. The solution was filtered through a 0.45 µm nylon syringe filter prior to injection [6].

Results & Discussion

Method Development and Optimization

Chromatographic parameters including mobile phase ratio, pH, flow rate, and organic modifier were optimized to achieve sharp, symmetric peaks with satisfactory resolution, retention time, and tailing factor. Various buffer systems and organic solvents were tested to achieve system suitability parameters: theoretical plates >2000, tailing factor <1.5, and %RSD <2 [7].

Table 1: Optimized Chromatographic Conditions

Parameter	Condition
Column	Acquity UPLC BEH C18 (100 mm × 2.1 mm, 1.7 µm)
MP	0.1% OPA buffer**:** Acetonitrile (45:55 v/v)
FR	0.3 mL/min
Detection Wavelength	210 nm
Column Temp.	30°C
Injection Volume	2 µL
Run Time	4 min
Diluent	Water**:** Acetonitrile (50:50 v/v)

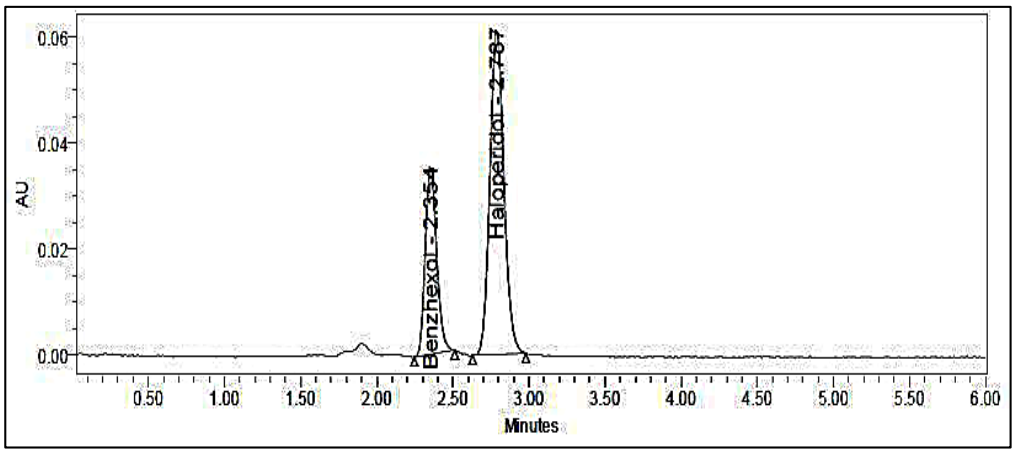

Fig 1: Optimized Chromatogram

Method Validation

The developed UPLC method was validated as per ICH Q2(R1) guidelines for the following parameters [8, 9]:

Specificity

Blank, placebo, standard, and sample solutions were injected to confirm the absence of interfering peaks. Both HPD and BZH exhibited high peak purity (purity angle < purity threshold), confirming that the method is specific and can accurately separate the drugs from excipients and degradation products.

Table 2: Specificity Results

Name of Solution	BZH RT (min)	HPD RT (min)
Blank	No peaks	No peaks
Standard	2.31	2.769
Sample	2.34	2.781

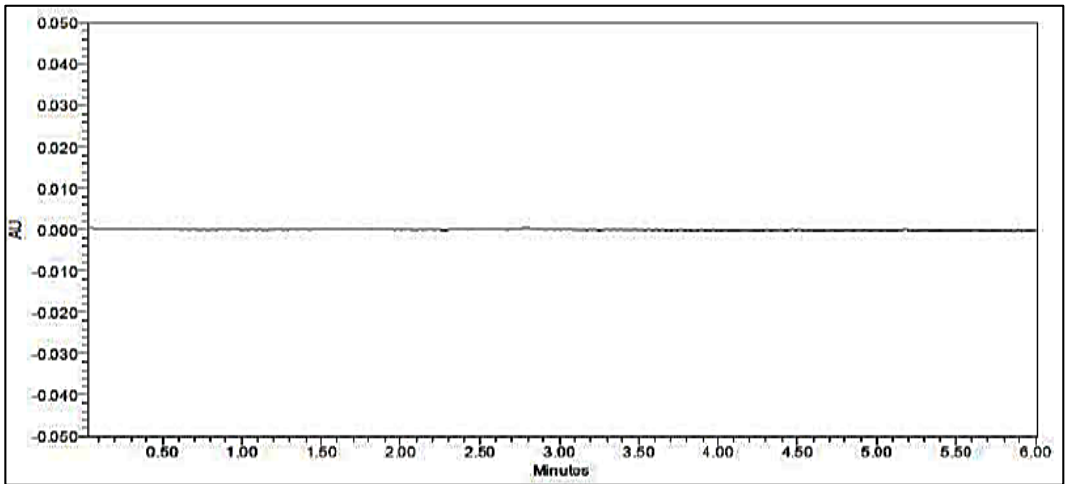

Fig 2: Chromatogram of Blank

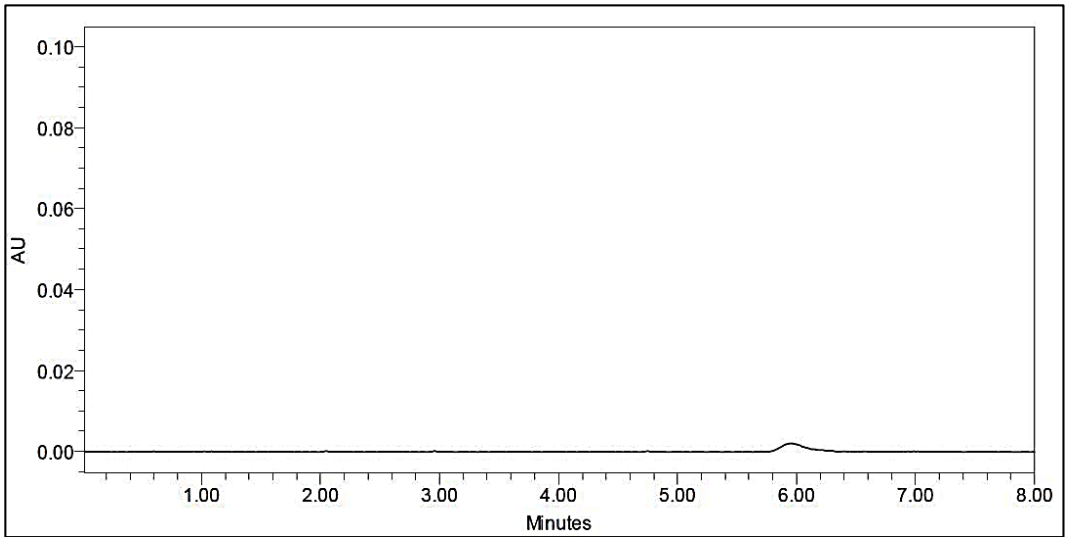

Fig 3: Chromatogram of Placebo

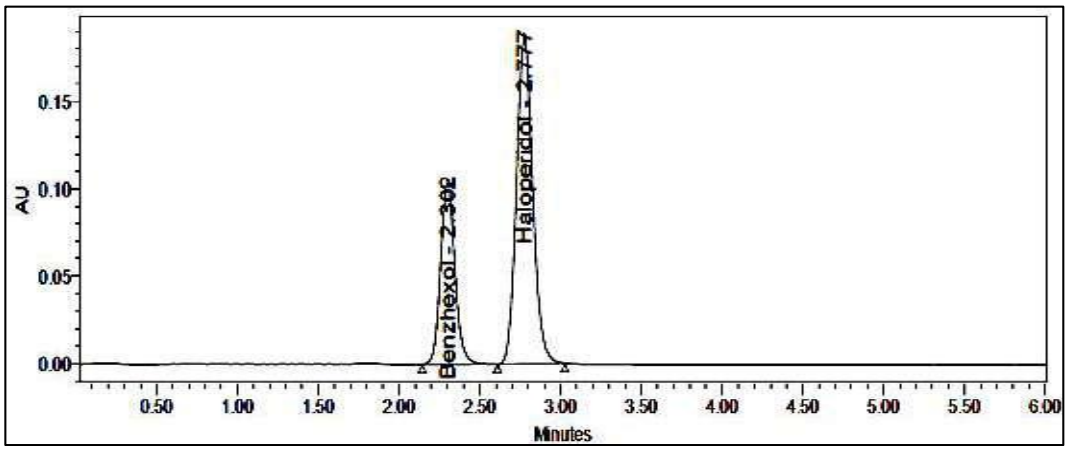

Fig 4: Chromatogram of Standard

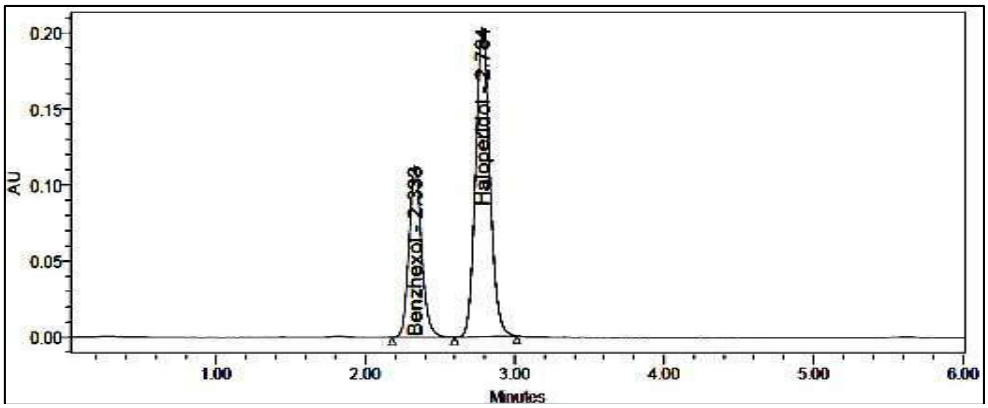

Fig 5: Chromatogram of Marketed Formulation

System Suitability Parameters

System suitability was evaluated by six replicate injections of standard solutions. Haloperidol showed a retention time (Rt) of 3.15 min and Benzhexol Rt of 1.87 min. Theoretical plates (N) were 4275 for HPD and 3980 for BZH, with tailing factors of 1.12 and 1.15, respectively. The %RSD for peak areas was <1.0% for both drugs, indicating the system was stable and within acceptance criteria.

Table 3: System Suitability Parameters

Name of Component	Peak Area	USP Plate Count	USP Tailing Factor	USP Resolution	%RSD
BZH	612890	3425	1.14	NA	0.68
HPD	133510	3845	1.09	2.82	1.21
Acceptance Criteria	NA	>2000	≤ 2	≥ 1.5	≤ 2

Degradation Studies

The stability-indicating nature of the method was confirmed under stress conditions:

- **Acidic Degradation:** HPD and BZH degraded 12% and 10%, respectively; peak purity remained intact.
- **Alkaline Degradation:** Degradation of 15% (HPD) and 13% (BZH); no co-eluting peaks observed.
- **Oxidative Degradation:** 10% degradation for HPD and 11% for BZH; peak purity confirmed.
- **Thermal Degradation:** Minor degradation (<5%) for both drugs; peak integrity maintained.
- **Photolytic Degradation:** 7% degradation for HPD and 6% for BZH; no interference detected.
- **Neutral (Water) Degradation:** <5% degradation; peak purity verified.

Table 4: Forced Degradation

Condition	HPD			BZH		
	% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold
Control	-	-	-	-	-	-
Acid	3.18	0.078	0.288	3.12	0.529	0.951
Alkali	2.63	0.118	0.296	2.95	0.507	0.559
Oxidation	1.81	0.085	0.311	1.82	0.369	0.617
Thermal	0.97	0.089	0.293	0.91	0.268	0.474
UV	0.84	0.084	0.299	0.69	0.213	0.468
Water	0.3	0.087	0.293	0.32	0.222	0.437

Overall, the developed UPLC method is specific, accurate, precise, robust, and stability-indicating for the simultaneous determination of Haloperidol and Benzhexol in pharmaceutical formulations.

Precision

Repeatability (intra-day) and intermediate precision (inter-day) were assessed. The %RSD for intra-day precision was 0.9% for HPD and 1.02% for BZH. Inter-day precision %RSD was 1.12% for HPD and 1.25% for BZH. These results were within the acceptance limit of ≤2%,

Table 5: Repeatability and intermediate precision

S. No	Repeatability (Intraday)		Intermediate (Interday)	
	BZH Area	HPD Area	BZH Area	HPD Area
1	612480	1318125	632540	1314260
2	614935	1325420	624880	1329785
3	607845	1332140	634220	1315425
4	608325	1343255	638540	1338620
5	616150	1329520	619980	1303580
6	614210	1307425	624520	1345925
Average	612991	1325981	629780	1326266
Std Dev	3895.6	13315.2	7018.5	17250.4

% Assay

The assay of the pharmaceutical formulation revealed that BZH content ranged from 99.43% to 101.02%, while HPD ranged from 99.6% to 101.1%, with an average assay of 100.65% for BZH and 100.15% for HPD.

Table 6: System and Method precession data (%Assay of Formulation)

S.no	System precession data		Method precession data		% Assay of BZH	% Assay of HPD
	BZH Std Area	HPD Std Area	BZH sample Area	HPD sample Area		
1	609210	1321458	611025	611025	100.09	99.89
2	613105	1351980	616020	616020	100.99	99.6
3	611320	1332456	607130	607130	99.49	100.3
4	613075	1316420	606895	606895	99.43	101.1
5	604085	1312796	616315	616315	100.95	100
6	604260	1349654	616682	616682	101.02	101

Linearity and Range

Calibration curves were constructed over the ranges of 50-300 µg/mL for HPD and 10-60 µg/mL for BZH. Linear regression analysis yielded correlation coefficients (r^2) of 0.9996 for HPD and 0.9994 for BZH, demonstrating excellent linearity within the studied concentration ranges.

Table 7: Linearity Data for BZH and HPD

BZH		HPD	
Conc. (µg/mL)	Peak Area (BZH)	Conc. (µg/mL)	Peak Area (HPD)
25	150125	25	385412
50	292880	50	721960
75	441205	75	1039420
100	602315	100	1354328
125	743112	125	1695184
150	868425	150	2022105
Correlation coefficient 0.99		Correlation coefficient 0.99	

Accuracy

Accuracy was evaluated at 80%, 100%, and 120% spiking levels. The %recovery for HPD ranged from 98.75-101.32% and for BZH from 98.62-101.15%. The %RSD values were <2%, confirming the method is accurate and meets ICH acceptance limits.

Table 8: Recovery Data for BZH

Conc. Level (%)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean % Recovery	% RSD
50%	10	9.85	98.5	99.36	0.8
		9.91	99.1		
		9.96	100.49		
100%	20	20.3	101.5	100.58	0.78
		20.05	100.25		
		19.8	99.99		
150%	30	30.45	101.51	99.83	1.02
		29.95	99.83		
		29.7	98.14		
Overall % Recovery = 99.92%					

Table 9: Recovery Data for HPD

Conc. Level (%)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean % Recovery	% RSD
50%	50	49.5	99	99.85	0.84
		50.8	101.6		
		49.7	99		
100%	100	100.5	100.5	100.96	0.68
		101.2	101.2		
		101.1	101.2		
150%	150	148.8	99.2	99.6	0.61
		149.5	99.7		
		150.6	100.4		
Overall % Recovery = 99.94%					

LOD & LOQ

LOD and LOQ were determined using the standard deviation and slope method. LOD values were 0.02 µg/mL for HPD and 0.01 µg/mL for BZH. LOQ values were 0.05 µg/mL for HPD and 0.03 µg/mL for BZH. These low values indicate the method is highly sensitive.

Table 10: Sensitivity Data of BZH and HPD

Drug Name	LOD (µg/mL)	LOQ (µg/mL)
BZH	0.01	0.04
HPD	0.91	2.76

Method Robustness

Standard and sample solutions were stable at room temperature and in the autosampler for 48 hours, with assay deviations ≤2% and unchanged peak purity.

Table 11: Robustness Results for BZH and HPD

S.No	Parameter	Optimized	Varied	BZH Peak Area	RT*	%RSD	HPD Peak Area	RT*	%RSD
1	Flow Rate	1	1.1	615432	2.12	1.1	12498560	2.57	1.6
			0.9	702185	2.59	0.7	14629542	3.1	0.4
2	Mobile Phase	45:55:00	50:60	679245	2.33	0.8	13911245	2.8	0.5
			40:50:00	687921	2.3	1.6	14218976	2.76	1.1
3	Temperature	30°C	35°C	671548	2.32	0.5	13951268	2.78	0.6
			25°C	700356	2.55	0.7	14701532	3.12	0.4

Conclusion

The developed RP-HPLC/UPLC method for simultaneous estimation of Haloperidol (HPD) and Benzhexol (BZH) is simple, rapid, precise, and accurate. Validation parameters including linearity, accuracy, precision, robustness, LOD, and LOQ were within ICH acceptance limits, confirming method reliability. Forced degradation studies demonstrated the stability-indicating capability, showing that the method can effectively separate degradation products from the intact drugs. The assay results (BZH: 99.43-101.02%; HPD: 99.6-101.1%) further confirm the method's suitability for routine quality control and stability testing of both bulk drugs and combined pharmaceutical formulations.

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