

**Validated Stability Indicating HPTLC, UHPLC and UV-Spectrophotometric Techniques for the Determination of Bepotastine Besilate in presence of its oxidative degradate**

**Abstract**

Comment [WU1]: Edited, made continuous

The study is aimed at developing methods which has a complete validation as stipulated in the ICH guidelines and to be applied for the determination of Bepotastine besilate (BB) in pure form and in pharmaceutical formulations in the presence of its oxidative degradation product.

High performance thin layer chromatography (HPTLC), Ultra high performance liquid chromatography (UHPLC) and different spectrophotometric methods (first derivative, first derivative of ratio spectra and ratio difference are developed for simultaneous determination of bepotastine besilate in laboratory-prepared mixtures of bepotastine besilate with its oxidative degradate and in pharmaceutical formulations were used in the study design.

Firstly, HPTLC was performed and separation occurred on silica gel 60 F254 plates, with butanol: ammonia (8:2, v/v) as a developing system. Secondly, was UHPLC in which separation occurred on a Kinetex C 18 column using methanol- 0.1% O-phosphoric acid - acetonitrile (70:20:10, by volume) as mobile phase. And lastly was uv-spectrophotometry which included first derivative determination of the drug at 252.6 nm, first derivative of ratio of peak amplitudes at 233.4, 250 and 275.6 nm and the ratio difference with the amplitude difference between (240 nm and 260 nm). Result showed that HPTLC method was applicable over the concentration range of 0.5-5 µg / band, while UHPLC method was linear over the concentration 2- 12 µg/mL and spectrophotometric methods were linear over the concentration range 20-120 µg/mL for bepotastine besilate. The proposed three techniques are quite accurate and precise. They can be used for routine analysis of bepotastine besilate in pharmaceutical formulation and stability indicating methods.

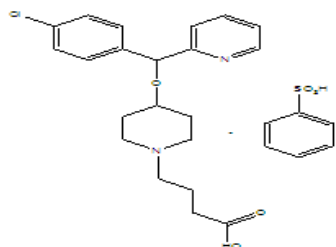
**Keywords:** Bepotastine besilate; UHPLC; HPLC; uv-spectrophotometry; oxidative degradate; Stability

Comment [WU2]: Edited, some key words added

**1. Introduction**

Bepotastine besilate (Bepotastine-B) is 2nd generation antihistamine and is chemically benzenesulfonic acid; 4-[4-[(S)-(4-chlorophenyl)- pyridin-2-ylmethoxy]piperidin-1-yl]

butanoic acid<sup>[1]</sup>. It is soluble in acetonitrile and methanol. It was approved in Japan for use in the treatment of allergic rhinitis and urticaria/pruritus. It is a direct H1 receptor antagonist that inhibits the release of histamine from mast cells<sup>[2]</sup>. Literature survey revealed that few methods have been reported for its determination in pharmaceutical formulations and biological samples including RP-HPLC techniques<sup>[3,4]</sup>, LC-MS/MS<sup>[5]</sup> method for determination of the drug in human plasma and urine where sample was prepared by solid phase extraction using mobile phase of acetonitrile: water: 200mmole/L ammonium formate (75:20:5) at flow rate 0.3 mL/min, 30°C and electrospray ionization mass spectrometry (MRM) detection and stability indicating HPTLC<sup>[6]</sup> determination of Bepotastine-B in presence of its acid degradate using chloroform: methanol (5: 5) as mobile phase where Bepotastine-B and acid degradate were obtained at Rf 0.50 and 0.78, respectively with UV detection at 225 nm. The acid degradate was undescribed in details. None of these methods is concerned with the identification and elucidation of the structure of the resulting oxidative degradation product, thus the objective of the present study was to develop simple and accurate stability -indicating methods for selective determination of Bepotastine-B in the presence of its oxidative degradation product.



**Fig. (1):** Chemical structure of bepotastine besilate

## 2. Experimental

### 2.1 Instrumentation

The UHPLC system used was an Agilent 1100 UPLC with binary pump and UV detector, analysis was performed on a Kinetex C 18 column (100 mm, 4.6 mm i.d., 2.6  $\mu$ m) ; Torrance, USA. Merck TLC plates used were 20 x 20 cm precoated with silicagel 60 F 254 (Fluka, Chemie, Switzerland), a camag Linomate 5 sample applicator equipped with a 100

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$\mu\text{L}$  syringe (Hamilton, Germany) 20 x 20 cm twin through glass chamber (Camag). The plates were scanned with a camag TLC scanner 3 with WINCATS computer software (Switzerland) using UV lamp with short wavelength (254 nm) (Desega- Germany). Shimadzu UV/Vis spectrophotometer (PC – 1601, Tokyo, Japan), using 1.0 cm quartz cells. Scans were carried out in the range from 200–400 nm at 0.5 nm intervals. Spectra were automatically obtained by Shimadzu UV-Probe 2.32 system software.

## **2.2. Materials and solvent**

Pure samples: Bepotastine-B was kindly supplied by RAMEDA Co., Giza, Egypt. Its purity was 99.80% as stated by the supplier. Market samples: Bepogra<sup>®</sup> tablets, B.N. (J701271) labelled to contain 10 mg Bepotastine -B (Lupin LTD, Jammu, India).

Solvents: Hydrogen peroxide (30%), Butanol and ammonia were obtained from El-Nasr Co., Qalyubia, Egypt. Methanol and O-Phosphoric acid were obtained from Sigma Aldrich (Schnelldorf, Germany) and Acetonitrile HPLC grade was obtained from Fisher (Loughborough, UK).

### **2.2.1 Preparation of Standard solutions**

Stock solution of Bepotastine-B (1 mg/mL) was prepared in methanol. Working solutions were freshly prepared by suitable dilution of stock solution with methanol to obtain a concentration of 0.1 mg/mL drug.

### **2.2.2 Preparation of degradation product**

Accurately weighed 100.0 mg of intact Bepotastine-B was dissolved in 25 mL 10%  $\text{H}_2\text{O}_2$  and kept at room temperature for two days then allowed to dry at room temperature. Residue was extracted with methanol, applied as a band on preparative TLC plate and developed in the mobile phase. The band corresponding to oxidation product was scratched, dissolved in methanol and evaporated. The isolated oxidative degradate solid was used to prepare the stock solution and identified by mass spectral analysis.

## **2.3. Procedures**

### **2.3.1. Linearity**

**HPTLC method:** Different volumes of stock standard solution (1 mg/mL) containing 0.5-5.0 mg Bepotastine-B were introduced into a series of 10- mL volumetric flasks, and then volume was completed with methanol. Ten  $\mu\text{L}$  from each solution was applied to the TLC plates of silica gel 60 F254 plates. The plates were washed with the mobile phase consisting of butanol: 33% ammonia (8:2, v/v) were applied to pre-washed activated plates, as 6-mm

bands, 6 mm apart, by means of a Camag Linomat IV automated spray-on band applicator equipped with a 100- $\mu$ L syringe. The plates were developed with the mobile phase in a Camag twin-trough chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were removed and air dried. Densitometry was performed at 266 nm in reflectance mode with slit dimensions of 6.00 mm  $\times$  0.3 mm and scanning speed of 20 mm/s. Peak area of each concentration was then plotted against its corresponding drug concentration and regression equation was computed.

**UHPLC method:** Aliquots of working standard drug solution (0.1 mg /mL in methanol) containing 0.02-0.12mg drug were introduced into series of 10- ml volumetric flasks and adjusted to the volume with methanol. Triplicate 10 $\mu$ L injections were made for each concentration on a C18 column followed by elution with a mobile phase of methanol- 0.1% O-phosphoric acid - acetonitrile (70:20:10, by volume) at a flow rate of 1 mL/ min and UV detection at 260 nm was used for detection.. The Peak area of each concentration was then plotted against the corresponding drug concentration and regression equation was computed.

**Spectrophotometric methods:** Aliquots equivalent to 0.2-1.20 mg/mL Bepotastine-B were accurately transferred from its standard solution (0.1 mg/mL) into a series of 10 -mL volumetric flasks then completed to volume with methanol. The spectra of the prepared solutions were scanned from 200 - 400 nm and stored in the computer.

**First derivative (1D) method:** 1D spectra of both Bepotastine-B and its oxidative degradate were recorded against methanol as blank, where the zero crossing wavelengths at 252.6 nm was selected for the analysis of Bepotastine-B in presence of its oxidative degradate. The calibration curve was constructed and the regression equation was calculated.

**Derivative ratio (1DR) method:** The stored spectra of Bepotastine-B was divided by the spectrum of 40 $\mu$ g/mL of its oxidative degradate. Then the first derivative of the ratio spectra (1DR) with scaling factor 10 is obtained and smoothed with  $\Delta\lambda = 8$  nm. 1DR signals at 233.4nm, 250nm and 275.6 were measured and then plotted against their corresponding concentration from which regression equations were computed.

**Ratio difference (RD) method:** The above procedure detailed under derivative ratio (1DR) method was followed and after the division of the stored spectra of Bepotastine-B by the spectrum of 40 $\mu$ g/mL oxidative degradate, the amplitude difference between 240 nm and 260 nm for spectrum was plotted against its corresponding drug concentration and the regression equation was evaluated.

### 2.3.2. Application to laboratory prepared mixtures

**HPTLC method:** Aliquots equivalent to 4.5-0.5 mg of Bepotastine-B from its stock standard solution (1 mg/mL) were transferred into series of 10 ml volumetric flasks containing aliquots of its oxidative degradate derived from 0.5-4.5 mg intact drug and then diluted to the volume with methanol.

**UHPLC method:** Aliquots equivalent to 0.09-0.01 mg of Bepotastine-B from its working standard solution (0.1 mg /mL) were transferred into series of 10 mL volumetric flasks containing aliquots of its oxidative degradate derived from 0.01-0.09 mg intact drug and then diluted to the volume with methanol.

**Spectrophotometric method:** Aliquots equivalent to 0.9-0.1 mg of Bepotastine-B from its working standard solution (0.1 mg /mL) were transferred into series of 10 ml volumetric flasks containing aliquots of its oxidative degradate derived from 0.1-0.9 mg intact drug of degraded solution and then diluted to the volume with methanol.

The procedures detailed under linearity were followed for the three techniques and concentration of intact drug was calculated from corresponding regression equation.

Comment [WU4]: Misplaced ?

### 2.3.3. Application to pharmaceutical formulation

Ten Bepogra<sup>®</sup> tablets were accurately weighed and finely powdered. A weight equivalent to 100mg of Bepotastine-B was sonicated with about 30 mL of methanol in 100 ml volumetric flask for 20 minutes then the volume was completed with methanol to obtain a solution labeled to contain 1mg/mL Bepotastine-B. The solution was filtered and used to analyze the drug by HPTLC as detailed under linearity. The filtrate was diluted with methanol to obtain solution labeled to contain 0.1 mg/mL Bepotastine-B to be analyzed by UHPLC and UV-spectrophotometry as described under linearity.

## 3. Results and Discussion

### 3.1. Forced degradation

Stability of Bepotastine-B was studied under different stressed conditions. It was found that it was stable to acidic and alkaline hydrolysis upon refluxing with 5N HCL and 5N NaOH for 6 h while it is liable to oxidative degradation upon keeping with 10% H<sub>2</sub>O<sub>2</sub> at room temperature for two days. The obtained oxidative degradation product was separated on preparative TLC plates using butanol: 33% ammonia (8:2, v/v) as a developing system and its structure was identified by mass spectroscopy. Where its oxidative degradate showed main molecular ion peak at 581.45 m/z, whereas intact Bepotastine-B molecular ion at 549.45m/z,

which are equivalent to their molecular weights this indicate increasing in molecular ion peak equal 32 unit, Fig. 2. This suggested the addition of two oxygen to each nitrogen in both piperidine and pyridine rings. Thus, a proposed pathway for this oxidative degradation was illustrated in Scheme (1).

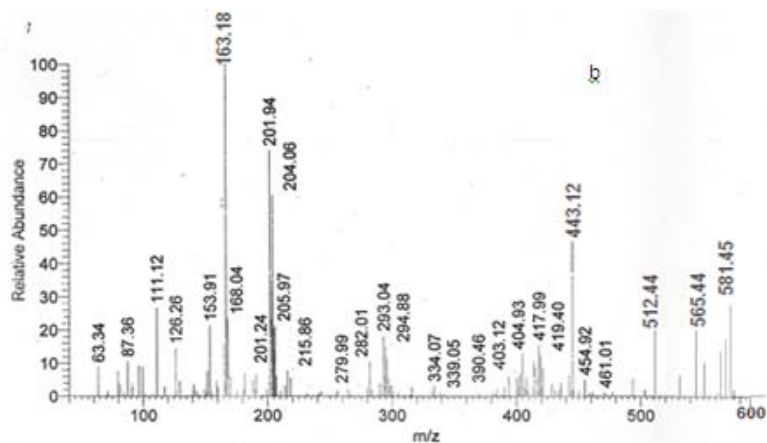
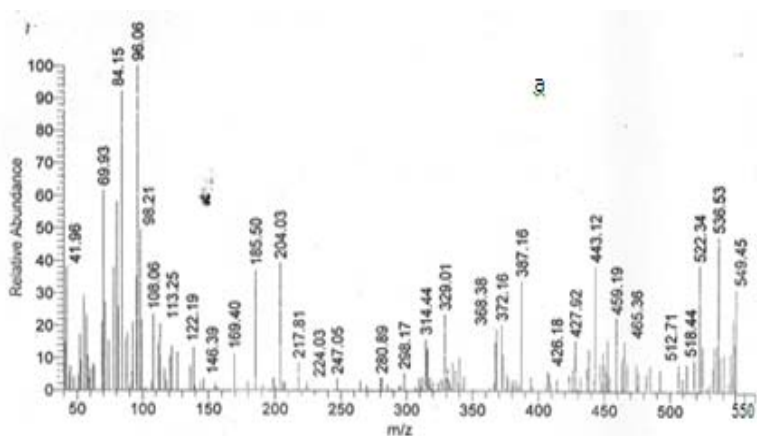
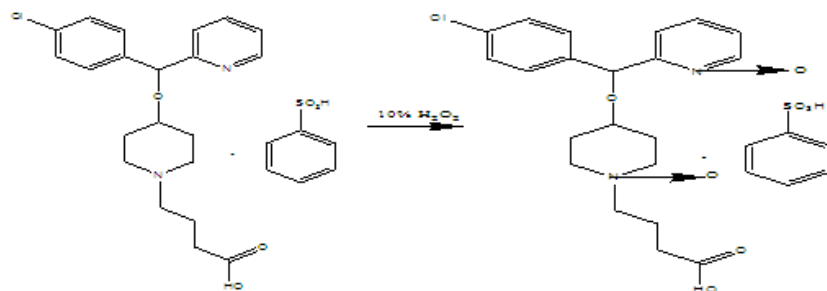


Fig. (2): Mass spectra of (a) intact Bepotastin besilate and (b) its oxidative degradate.

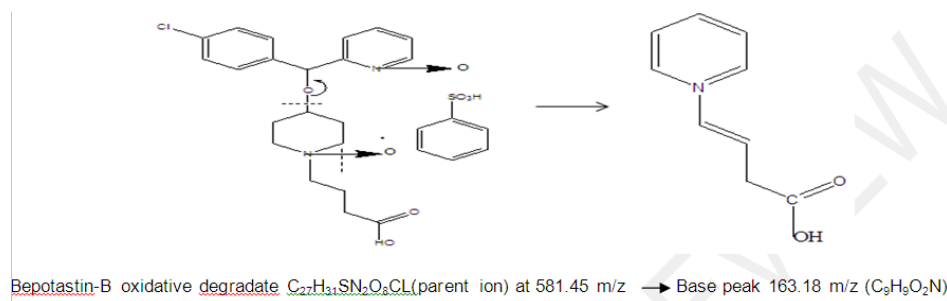
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**Scheme(1):** Suggested oxidative degradation pathway of Bepotastin besilate

The drug does not degrade upon treatment with  $H_2O_2$  but it undergoes oxidation of both nitrogen atoms due to presence molecular ion peak (parent ion) at 581.45 m/z corresponding to its molecular weight. However when the vaporized drug passes into ionization chamber of mass spectrum it is bombarded by a stream of electrons which break it to smaller fragments. The base peak 163.18 m/z may be due to fragmentation of the parent ion to give the most stable ion at 163.18 m/z which has molecular formula  $C_9H_9O_2N$ . This fragmentation was illustrated in Scheme (2) where piperidine ring stabilize itself to more stable pyridine ring.



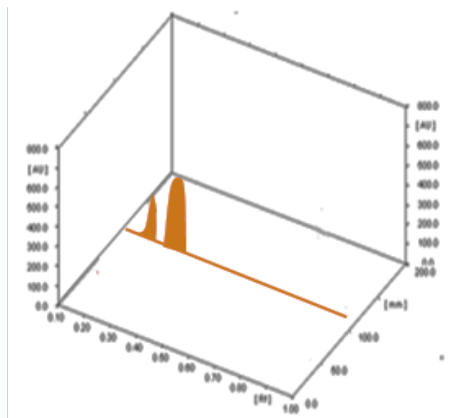
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**Scheme(2):** Fragmentation of Bepotastin besilate oxidative degradate

### 3.2. HPTLC method

Different mobile phases in different ratios and at different  $\lambda_{max}$  for detection were tried. It was found that butanol: ammonia 33% (8:2, v/v) as a developing system followed by

densitometric determination at 266 nm offered best separation and resolution where  $R_f$  were 0.35 and 0.26 for Bepotastine-B and its oxidative degradate, respectively, Fig.(3).



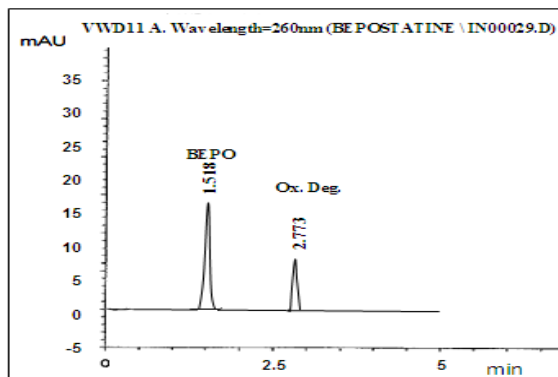
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**Fig. 3:** Densitogram of bepotastine-besilate (2.5  $\mu\text{g}/\text{mL}$ ) and its oxidative degraded derived from intact drug (2.5  $\mu\text{g}/\text{mL}$ ).

### 3.3. UHPLC method

Ultra Performance Liquid Chromatography (UHPLC) is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-2 $\mu\text{m}$  particles for stationary phase. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. Owing to its speed and sensitivity, this technique is gaining considerable attention in recent years for pharmaceutical and biomedical analysis<sup>[7]</sup>. Chromatographic separation of Bepotastine-B and its oxidative degradate using UHPLC were optimized. Different mobile phases in different ratios were studied, where best peak shape and adequate separation was obtained by using methanol- 0.1% O-phosphoric acid – acetonitrile (70:20:10, by volume). Different flow rates and wavelengths were tried; good resolution with sensitive detector response was obtained at 260 nm using a flow rate of 1  $\text{mL min}^{-1}$  to obtain a retention time 1.518 min for Bepotastine-B and 2.773 min for its oxidative degradate, respectively, Fig.(4).



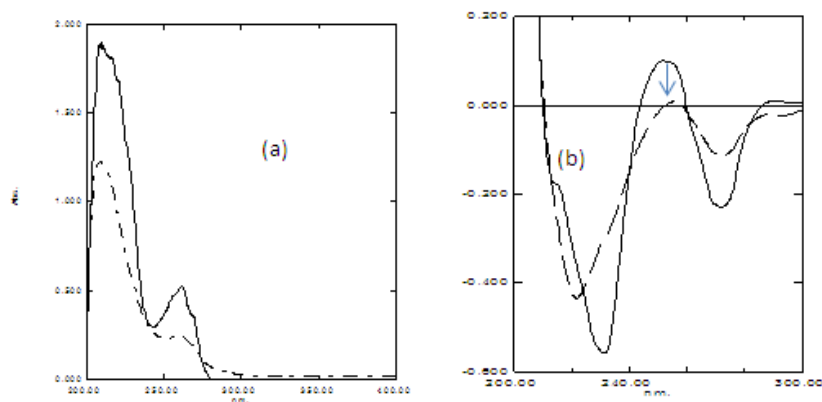


**Fig. 4:** UHPLC chromatogram of bepotastine-besilate ( 6  $\mu\text{g}/\text{mL}$ ) and its oxidative degradate derived from intact drug ( 4  $\mu\text{g}/\text{mL}$ ).

### 3.4. Spectrophotometric method

The zero-order absorption spectra of Bepotastine-B and its oxidative degradate showed severe overlapping over the entire spectrum of the intact drug; Fig. (5a). Therefore, the use of direct absorbance measurements for assaying Bepotastine-B in the presence of its degradation product was not possible.

**First-derivative (1D) method:** It was found that the above overlapping were somewhat resolved in 1D spectra and Bepotastine-B can be determined at 252.6 nm, at which its degradate has no contribution (zero crossing); Fig. (5b).

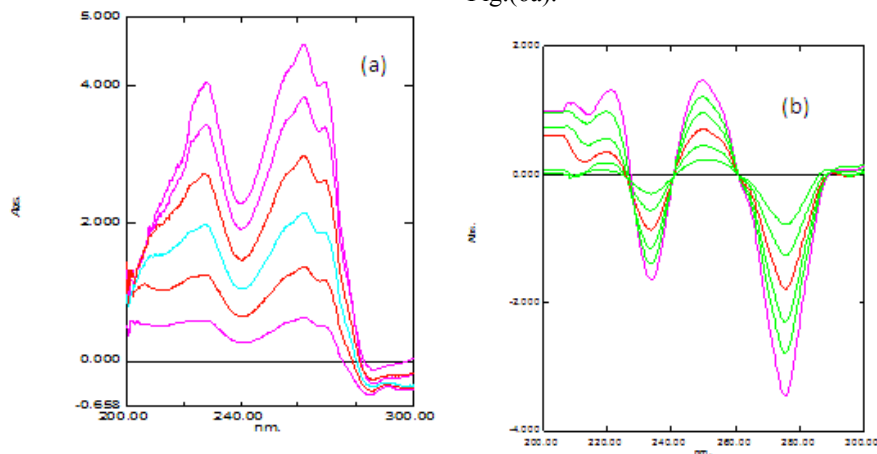


**Fig. 5:**(a) zero order spectra and (b) first order derivative spectra of  $40 \mu\text{g mL}^{-1}$  Bebotastine besilate (—) and its oxidative degradate derived from  $40 \mu\text{g mL}^{-1}$  intact drug (---).

**Comment [WU9]:** Incomplete and unclear caption and figure

**Derivative ratio (1 DR) method-** In this method the absorption spectrum of Bebotastine-B is divided by the absorption spectrum of oxidative degradate and the first derivative of the ratio spectrum is obtained. Different concentrations of oxidative degradate were tried as a divisor (20-120  $\mu\text{g/ml}$ ) and a divisor concentration of oxidative degradate derived from  $40 \mu\text{g/ml}$  Bebotastine-B was the best regarding average % recovery. At wavelengths 233.4, 250 and 275.6 nm using  $\Delta\lambda = 8 \text{ nm}$  and scaling factor 10, good linearity and recovery percent were observed; Fig. (6b). Consequently, the peaks amplitudes of 1DR were then recorded at 233.4, 250 and 275.6 nm.

**Ratio difference (RD) method-** This method comprises two critical steps. The first is the choice of the divisor and the selected divisor should compromise between minimal noise and maximum sensitivity. The second is the choice of the wavelengths at which measurements are recorded [8]. Linear correlation was obtained between the differences in amplitudes between 240 and 260 nm against the corresponding concentration of Bebotastine-B using  $40 \mu\text{g/ml}$  of oxidative degradate as divisor; Fig.(6a).



**Fig. 6:** (a) Ratio spectra and (b) First derivative of smoothed ratio spectra of Bebotastine besilate (20-120 $\mu\text{g/mL}$ ) using  $40 \mu\text{g/mL}$  oxidative degradate as divisor

### 1.1. Method Validation<sup>[9]</sup>

**Comment [WU10]:** Not acceptable format. Write the methodology adopted in text e.g. 'The procedural approach followed for method validation is that reported by [9]'

**System suitability:** System suitability test was performed in accordance with USP<sup>[10]</sup> to ensure system performance before or during the drug analysis. Results shown in Table 1 indicated adequate resolution.

**Table 1: System suitability results of the UPLC method**

Parameter	Bepotastine-B	Reference value
Number of theoretical plates (N)	6711	The higher the value, the more efficient the column is
Resolution factor	17.19	>2
Capacity factor (K)	3.52	1–10
Selectivity factor	8.20	≥1

**Linearity:** Under the described experimental conditions, linear calibration curves between peak areas to respective drug concentrations were obtained through the concentration ranges of 0.5 -5 µg/ spot and 2-12 µg/ mL and 20-120 µg/mL of Bepotastine-B using HPTLC, UHPLC and spectrophotometric methods, respectively. Regression parameters were computed, where values of  $r^2$  ranged between 0.9993 and 0.9999 indicating good linearity.

**Accuracy and precision:** Accuracy calculated as (R%) ranged from 99.67 to 100.58% for Bepotastine-B. Intraday precision RSD % ranged from 0.15 to 1.84%, while inter day precision ranged from 0.19 to 1.93%; indicating good repeatability and reproducibility of the proposed methods, Tables 2.

**Selectivity:** The selectivity of the proposed methods was assured by application to laboratory prepared mixtures of the intact drug together with its oxidative degradate. They were successfully applied for the determination of Bepotastine-B in the presence of up to 70% of its oxidative degradate for UHPLC and HPTLC methods and up to 60% for spectrophotometric methods with mean % recovery of 99.52±0.65 to 101.48±1.63 , Table 3.

## 1.2. Application to pharmaceutical formulation

The proposed techniques were successfully applied for analysis of Bepotastine-B in the Bepogra<sup>®</sup> Tablets with mean % recovery of 99.11±0.57 to 100.90±1.34. The validity of the proposed method was further assessed by applying the standard addition technique. The results obtained were reproducible with acceptable SD, Table (4). Statistical analysis of the results obtained by the proposed methods compared with a reported HPTLC method<sup>(6)</sup> showed

that the calculated t and F values were less than the tabulated ones indicating no significant difference between them confirming accuracy and precision at 95% confidence limit, Tables (5). The two chromatographic methods were more sensitive, less time and solvent consuming. While UV-spectrophotometric methods are cheap and easily available instrument. Therefore, should be cost-effective for routine analysis in the pharmaceutical industry.

**Table 2:** Regression and validation parameters for the determination of Bepotastine-Besilate by the proposed methods

Comment [WU11]: Try to proportionate all tables

	IPTLC	HPLC	(ID) First-derivative method	IDR method			RD method
λ max (nm)	266	260	252.6	233.4	250	275.6	240-260
Linearity range	0.5-5 spot	μg/ 2-12μg/ mL	20-120μg/ mL				
Regression parameters							
Slope (b)± SD	3004.5±12.476	25.784±0.004	0.003±2.67E-05	0.014±0.0002	0.012±0.0001	0.026±0.0004	0.018±0.0003
Intercept (a)± SD	115.73±37.858	0.004±0.0003	-	0.027±0.013	0.033±0.011	0.208±0.0003	-0.03±0.0203
Correlation coefficient (r <sup>2</sup> )	0.9999	0.9999	0.9996	0.9994	0.9995	0.9995	0.9993
Accuracy (R %)(n=9)	99.86	99.95	99.69	99.85	100.15	100.58	99.67
Precision (RSD %)							
Intra day	0.31-0.99	0.15-0.33	0.95-1.84	0.44-0.92	0.93-1.56	0.32-0.64	0.27-0.49
Inter day (n=9)	0.57-1.47	0.19-0.62	1.42-1.93	0.74-1.78	1.25-1.84	0.60-1.30	0.65-1.19

**Table 3:** Determination of Bepotastine-Besilate in laboratory prepared mixtures with its oxidative degradate by the proposed methods.

Method	Intact μg mL <sup>-1</sup>	Degradate μg mL <sup>-1</sup>	%Degradation	Recovery % of intact	Average recovery (mean ± SD)
HPTLC	4.5	0.5	10	101.21	100.09±0.97
	4	1	20	100.97	
	3.5	1.5	30	100.65	
	3	2	40	100.12	
	2.5	2.5	50	99.67	
	2	3	60	99.62	
	1.5	3.5	70	98.39	

UHPLC		9.5	0.5	5	100.37	99.52±0.99	
		9	1	10	100.24		
		8	2	20	100.23		
		7	3	30	100.08		
		6	4	40	99.59		
		5	5	50	99.22		
		4	6	60	99.03		
(1D) First-derivative method		3	7	70	97.41	100.85±1.45	
		95	5	5	98.75		
		90	10	10	99.30		
		80	20	20	100.14		
		70	30	30	101.75		
		60	40	40	101.42		
		50	50	50	102.44		
At 233.4 nm		40	60	60	102.13	101.31±0.65	
		95	5	5	100.86		
		90	10	10	100.90		
		80	20	20	100.93		
		70	30	30	100.96		
		60	40	40	101.12		
		50	50	50	101.78		
1DR method		40	60	60	102.59	100.84±1.63	
		95	5	5	99.39		
		90	10	10	99.44		
		80	20	20	99.63		
		At 250 nm	70	30	30		101.45
		60	40	40	101.74		
		50	50	50	103.39		
At 275.6 nm		40	60	60	103.02	101.48±1.17	
		95	5	5	100.15		
		90	10	10	100.37		
		80	20	20	100.51		
		70	30	30	101.67		
		60	40	40	101.83		
		50	50	50	102.80		
RD method		40	60	60	102.28	100.50±0.96	
		95	5	5	99.38		
		90	10	10	99.74		
		80	20	20	100.33		
		70	30	30	100.13		
		60	40	40	100.61		
		50	50	50	101.06		

**Table 4:** Application of standard addition technique for the determination of Bepotastine-Besilate in its pharmaceutical formulation by the proposed methods.

Method	Pharmaceutical formulations	Taken (ug mL <sup>-1</sup> )	Added (ug mL <sup>-1</sup> )	% recovery	Mean%±SD
HPTLC	99.57±0.57	2	0.5	98.21	100.22±1.49
			1	100.00	

				2	101.60	
				3	101.06	
				2	101.51	
<b>UHPLC</b>	99.91±0.69	4		4	100.50	100.72±0.53
				6	100.38	
				8	100.47	
<b>(1D) First-derivative method</b>	99.11±0.92	40		20	100.00	
				40	99.08	99.89±0.58
				60	100.00	
				80	100.46	
	At 233.4 nm			20	101.10	
				40	102.03	
	100.52±1.34		40	60	100.86	100.89±1.02
				80	99.55	
<b>IDR method</b>				20	100.38	
	At 250 nm			40	98.58	100.08±1.22
			40	60	99.86	
				80	101.51	
				20	98.30	
	At 275.6 nm			40	98.38	99.01±1.43
			40	60	98.22	
				80	101.15	
<b>RD method</b>	100.19±0.82	40		20	100.81	
				40	100.54	101.14±0.57
				60	101.45	
				80	101.77	

**Table 5:** Statistical analysis of the proposed analytical methods and reported method <sup>(6)</sup> for the determination of Bepotastine-Besilate in Bepogra<sup>®</sup> tablets

**Comment [WU12]:** Unacceptable. Put this in the discussion section

Parameter	HPTLC	UHPLC	(1D)	IDR method			RD method	Reported method <sup>(6)</sup>
			First-derivative method	233.4 (nm)	250 (nm)	275.6 (nm)		
Linearity	0.5-5 (µg/spot)	2-12 (µg/mL)	20-120 (µg/mL)					5-25 (µg/spot)
N	5	5	5	5	5	5	5	5
Mean%	99.57	99.91	99.11	100.52	100.90	100.02	100.19	99.90
±SD	±0.57	±0.69	±0.92	±1.34	±0.99	±1.09	±0.82	±0.57
Variance	0.32	0.48	0.85	1.80	0.98	1.19	0.67	0.32
t-	0.92	0.03	1.63	0.96	1.96	0.22	0.65	-
F-	1.00	1.47	2.61	5.53	3.02	3.66	2.07	-

**Comment [WU13]:** See my comment above

The theoretical t- and f- values at p= 0.05 are 2.31 and 6.39, respectively.

Reported method <sup>(6)</sup>involved stability indicating HPTLC method for estimation of Bepotastine-B. separation was carried out on Merck TLC aluminium sheets precoated with silica gel 60F254 using mobile phase as chloroform : methanol. (5:5 v/v) at 225nm.

#### **4. Conclusion**

The proposed three techniques are rapid, accurate and precise and can be used for the analysis of Bepotastine-B in pure form and in pharmaceutical formulations in the presence of its oxidative degradation product. The sample recovery for all five methods was in good agreement with their respective label claims which suggested no interference of additives and excipients.

#### **Competing interests**

Authors have declared that no competing interests exist.

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