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Development of Analytical Method to Monitor Dissolution of Bepotastine Besilate Tablet

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ABSTRACT

Bepotastine Besilate is an anti-histaminic drug and it is marketed as tablet of strength 10mg. In this study an attempt is made to monitor the dissolution of Bepotastine Besilate tablet. Dissolution study was done for marketed sample using phosphate buffer 6.8, phosphate buffer 4.5 and 0.1 N HCl as dissolution media. Samples were analysed using UV spectrophotometer, HPLC and HPTLC. Detection wavelength selected was 226nm. A chromatographic separation is achieved on a C18 column with a mobile phase consisting of acetonitrile, water with isocratic elution with flow rate 1ml/min. Solvents used for development in HPTLC were chloroform and methanol. Percentage release of bepotastine besilate was calculated by extrapolation of calibration curve. The results of three analytical methods were compared by applying One-Way ANOVA.

Keywords: Bepotastine Besilate, Dissolution, UV, HPLC, HPTLC

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INTRODUCTION

Bepotastine is a second-generation nonsedating antihistamine. It possesses a dual mode of action as it also stabilizes mast cell function and suppresses migration of eosinophils into the inflamed tissues[1]. Its molecular formula is $C_{21}H_{25}ClN_2O_3$. Chemically it is benzenesulfonic acid;4-[4-[(S)-(4-chlorophenyl)- pyridin-2-ylmethoxy]piperidin-1-yl]butanoic acid as shown in fig.1.

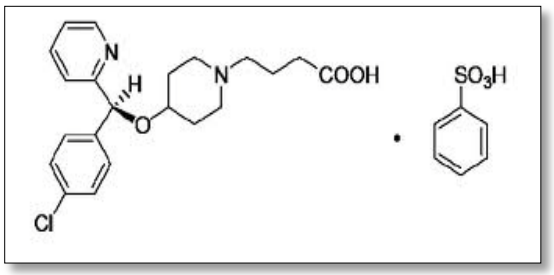

Fig 1: Structure of Bepotastine Besilate

Molecular weight is 547.063 g/mol[2]. 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 available as ophthalmic solution and oral tablet. It is a direct H1 receptor antagonist that inhibits the release of histamine from mast cells. Literature survey revealed the estimation of Bepotastine by several techniques such as simultaneous estimation RP-HPLC techniques[3,4], Stability indicating method by HPTLC[5], development of alternative salt i.e. Bepotastine salicylate[6], comparison of branded and generic[7].

MATERIAL AND METHOD

All AR grade chemicals and reagents i.e. Methanol, Chloroform, Acetone, Hydrochloric acid(HCL), Sodium hydroxide (NaOH), Potassium Dihydrogen Phosphate (KH_2PO_4) were purchased from LOBA CHEMIE PVT. LTD., Mumbai.

Selection of Wavelength

Standard stock solution of 1,000ug/ml was prepared by using ACN. Further dilution was carried out to make solution of 10µg/ml and was scanned over 200 to 400nm in UV-Spectrophotometer. Wavelength 226nm showed considerable absorbance hence it was selected as analytical wavelength. UV spectrum is given as

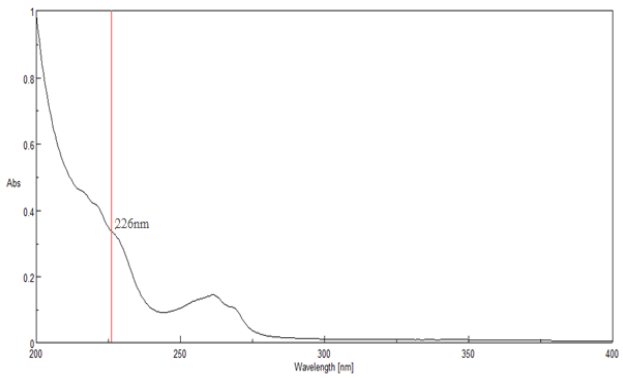

Fig 2: Spectrum of Bepotstine Besilate (10µg/ml in ACN)

Preparation of Stock Solution:

UV and HPLC- 10 mg of Bepotastine besilate was dissolved in 10 ml ACN to make 1000 µg/ml. Further dilutions were made in ACN to make 100 µg/ml. Further dilutions 3, 6, 9, 12, 15 µg/ml were made to take absorbance and peak area in UV-Visible spectrophotometer and HPLC respectively.

HPTLC- 10 mg of Bepotastine besilate was dissolved in 10 ml ACN to make 1000 µg/ml. Further dilutions were made in ACN to make 50 µg/ml. Solution from 50µg/ml applied on TLC plate to get 200 to 800ng/band.

Chromatographic Condition

HPLC

HPLC system used was Jasco consisting PU 2082 Plus pump, Rheodyne (Capacity – 50ul) injector, Jasco UV 2075 plus detector and software Borwin (Version – 1.5). The chromatographic separation was optimized using Nucleosil C18 column (250x4.6mm) and mobile phase was composed of Acetonitrile: Water (0.1ml OPA v/v is added in 100 ml water then pH is adjusted to 3 by Triethylamine) in proportion of 80:20v/v. The flow rate of the mobile phase and the column temperature was set as 1.0 ml/min and 45°C. The detection wavelength was done at 226 nm.

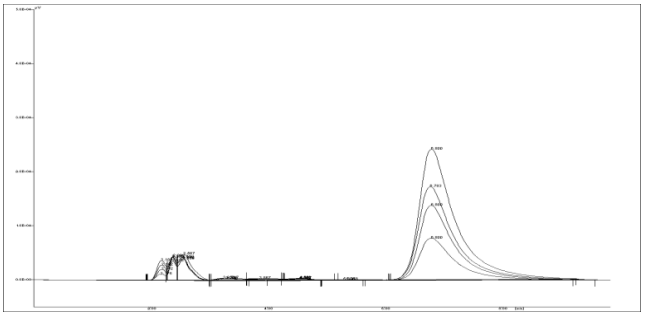

Fig 3: Chromatogram of standard linearity of Bepotastine Besilate (3, 6, 9, 12µg/ml in ACN)

HPTLC

Optimisation of chromatographic condition was carried out on aluminum plates precoated with silica gel $60F_{254}$ in (20 cm × 10 cm with 250µm layer thickness). Sample was applied on the plate as a band of 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with Linomat 5 applicator (Camag Switzerland). The mobile phase was composed of Chloroform: methanol 5:5v/v. Mobile phase was saturated in Camag (20cm × 10cm) twin trough glass chamber for 15 min. saturation condition and run to distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by winCATS (version 1.4.3.6336).

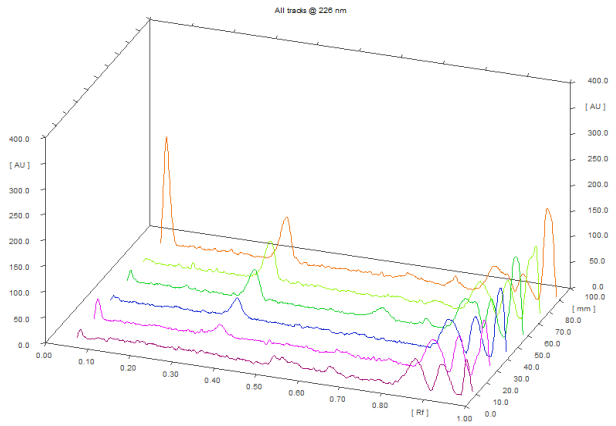

Fig 4: 3D Densitogram of Bepotastine Besilate (track 1 is blank ACN, track 2-6 linearity 200-800 ng/band)

Dissolution Studies

Preparation of Dissolution Media[8-11]:

- Buffer 6.8- 50ml of 0.2M KH_2PO_4 is mixed with 22.4 ml of 0.2M NaOH and volume was made upto 200ml
- Buffer 4.5- 6.8gm of KH_2PO_4 in 1000 ml water.
- 0.1 N HCl – 8.5ml of concentrated HCl in 1000ml water.

Procedure of Dissolution:

The dissolution test was performed on Dissolution Testing Apparatus 2 (paddle method) using 900 ml medium at 37 ±0.5°C and 50 rpm. Six marketed tablets of Bepotastine Besilate of strength 10 mg were added to six vessels. 10ml sample was withdrawn after 30 mins. Sample was filtered through whatman filter paper and monitored using UV-Visible spectrophotometer, HPLC, HPTLC. The procedure is repeated six times for each dissolution media.

RESULT AND DISCUSSION

1) Phosphate buffer 6.8

a) UV method

$y = 0.012x - 0.012$
$R^2 = 0.997$

Table 1: Percent release of BB in Phosphate buffer 6.8 by UV method

No. of flasks	% release
1	99.65
2	96.25
3	101.17
4	99.88
5	98.79
6	100.51

b) HPLC method

y = 58035x + 35550

R^2 = 0.997

Table 2: Percent release of BB in Phosphate buffer 6.8 by HPLC method

No. of flasks	% release
1	97.30
2	96.27
3	95.62
4	94.94
5	95.74
6	95.63

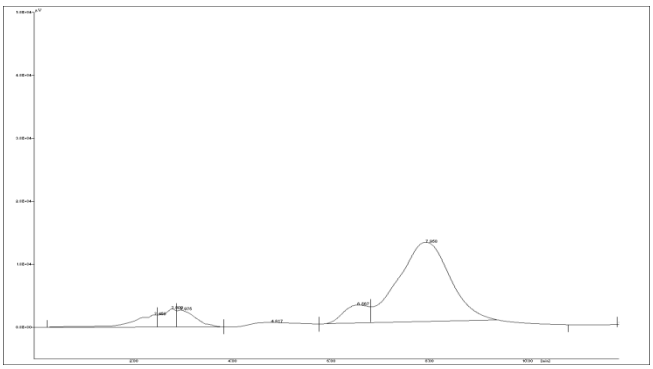

Fig 5: Chromatogram of Bepotastine Besilate in Phosphate buffer 6.8 (15µg/ml)

C) HPTLC method

y = 1.765x + 120.0 R^2 = 0.998

Table 3: Percent release of BB in Phosphate buffer 6.8 by HPTLC method

No. of flasks	% release
1	89.17
2	88.98
3	89.14
4	89.69
5	89.69
6	88.78

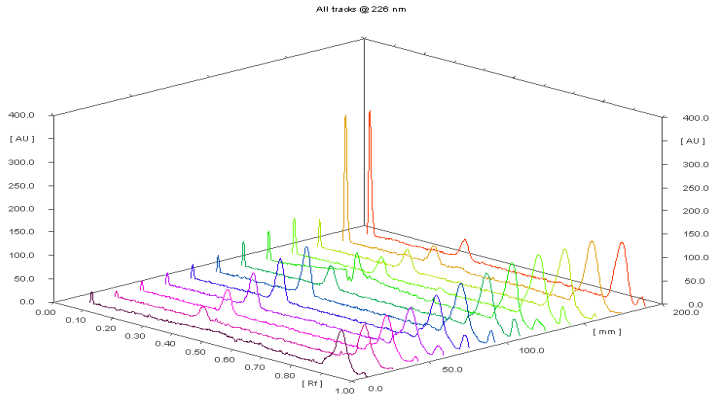

Fig 6: 3D Densitogram of standard linearity and dissolution samples of Bepotastine Besilate in Phosphate buffer 6.8(track 1 is blank ACN, track 2-6 linearity 200-800 ng/band, track 7-12 dissolution samples)

2) Phosphate buffer 4.5

a) UV method

y = 0.009x + 0.008
R^2 = 0.999

Table 4: Percent release of BB in Phosphate buffer 4.5 by UV method

No. of flasks	% release
1	100.00
2	100.34
3	98.42
4	100.68
5	98.91
6	97.69

b) HPLC method

y = 45009x + 87790
R^2 = 0.996

Table 5: Percent release of BB in Phosphate buffer 4.5 by HPLC method

No. of flasks	% release
1	95.08
2	95.93
3	92.46
4	95.42
5	94.64
6	93.95

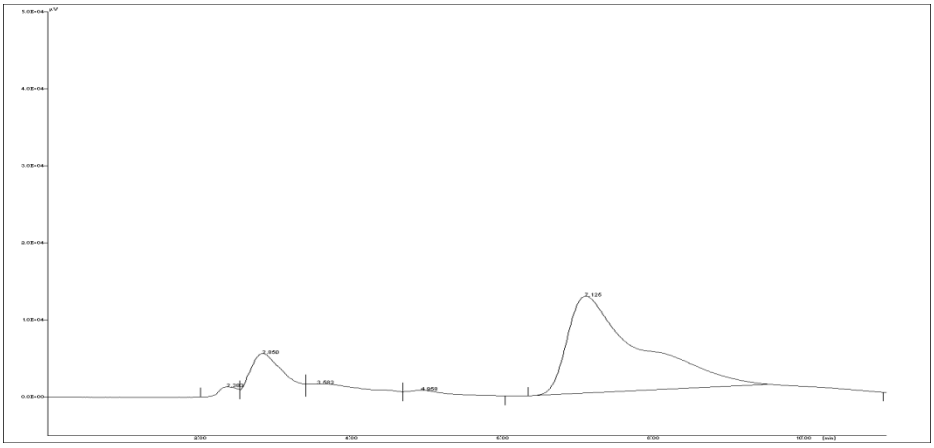

Fig 7: Chromatogram of Bepotastine Besilate in Phosphate buffer 4.5 (15µg/ml)

c) HPTLC method

y = 1.640x + 351.8
R^2 = 0.995

Table 6: Percent release of BB in Phosphate buffer 4.5 by HPTLC method

No. of flasks	% release
1	89.12
2	88.89
3	88.12
4	89.54
5	89.01
6	89.56

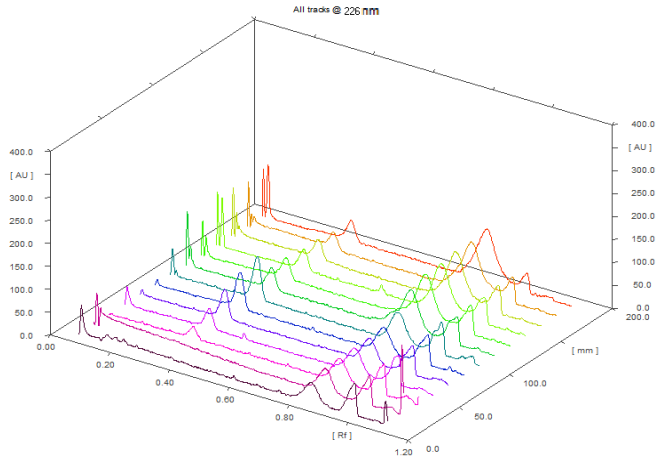

Fig 8: 3D Densitogram of standard linearity and dissolution samples of Bepotastine Besilate in Phosphate buffer 4.5 (track 1,2 is blank ACN, track 3-7 linearity 200-800 ng/band, track 8-13 dissolution samples)

3) 0.1 N HCl

a) UV method

y = 0.027x - 0.032
R^2 = 0.996

Table7: Percent release of BB in 0.1 N HCl by UV method

No. of flasks	% release
1	97.91
2	99.33
3	97.76
4	99.01
5	97.20
6	101.81

b) HPLC method

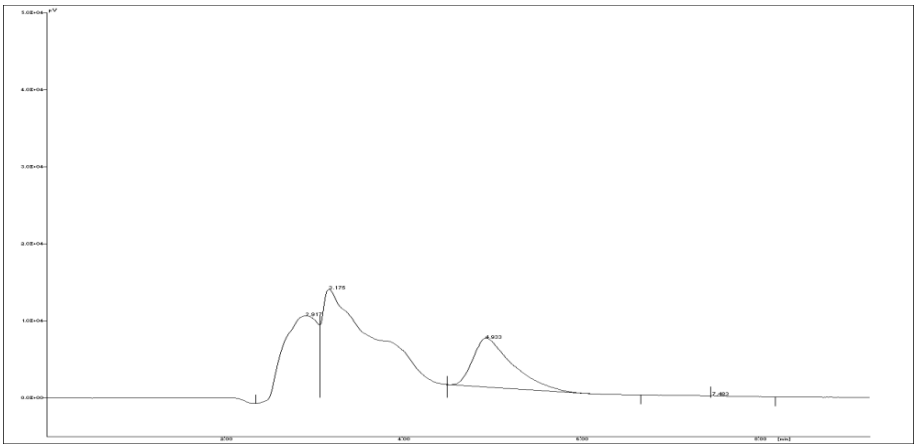

Fig 9: Chromatogram of Bepotastine Besilate in Phosphate buffer 0.1 N HCl(15µg/ml)

HPLC study of Bepotastine Besilate By using 0.1N HCl as dissolution medium showed that the degradation product of Bepotastine Besilate eluted at 4.46mins where as standard BB was expected to be eluted at 7 mins.

c) HPTLC method:

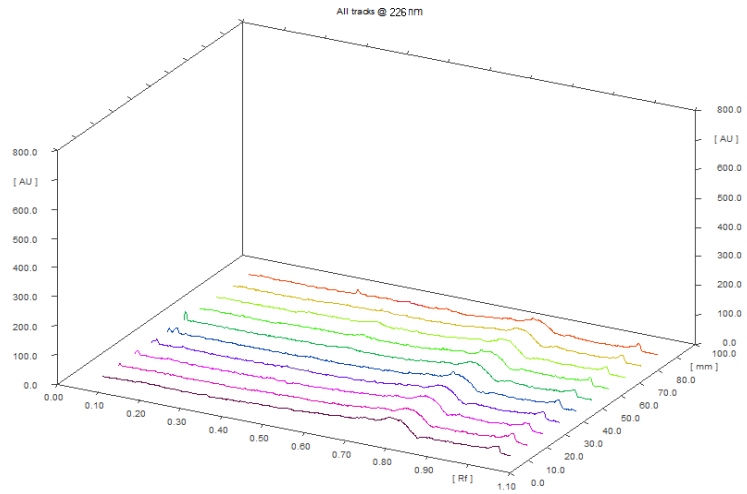

Fig 10: 3D Densitogram of linearity and dissolution samples of Bepotastine Besilate in 0.1 N HCl (track 1,2 is blank ACN, track 3-7 linearity 200-800 ng/band, track 8-10 dissolution samples)

HPLC study of Bepotastine Besilate By using 0.1N HCl as dissolution medium did not show any peak at Rf of bepotastine besilate.

Table 9: Percentage release of bepotastine besilate tablet

Media	Buffer 6.8			Buffer 4.5			0.1 N HCl		
No. of flasks	UV	HPLC	HPTLC	UV	HPLC	HPTLC	UV	HPLC	HPTLC
1	99.65	97.30	89.17	100.01	95.08	89.12	97.91	-	-
2	96.25	96.27	88.97	100.34	95.93	88.89	99.32	-	-
3	101.17	95.62	89.14	98.42	92.46	88.12	97.76	-	-
4	99.88	94.94	89.69	100.68	95.42	89.54	99.01	-	-
5	98.79	95.74	89.69	98.91	94.64	89.01	97.20	-	-
6	100.51	95.64	88.78	97.69	93.95	89.56	101.81	-	-
Mean	99.37	95.92	89.24	99.34	94.58	89.04	98.84	-	-
SD	1.73	0.80	0.37	1.18	1.24	0.53	1.60	-	-

Statistical Analysis

Statistical analysis was carried out using GraphPad Instat 3. All of the data is shown as the mean ± standard Deviation and were analysed using one-way analysis of variance (ANOVA). Significant differences between three analytical methods were determined using Tukey-Kramer multiple comparisons test, $P<0.05$ was considered significant.

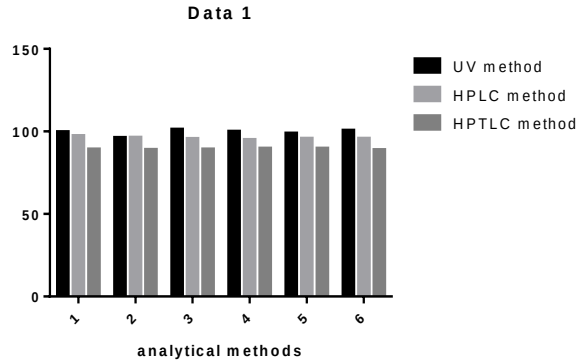

Fig 11: Graphical comparison of % release in phosphate buffer 6.8 by three analytical methods

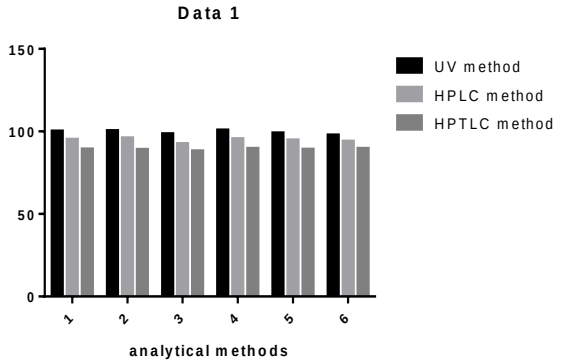

Fig 12: Graphical comparison of % release in phosphate buffer 4.5 by three analytical methods

Table 10: Comparison of % release by analytical methods to monitor dissolution in different dissolution media

Sr. No.	Dissolution media	UV method	HPLC method	HPTLC method
1	Phosphate buffer 6.8	99.37 ± 1.73	95.92 ± 0.79	89.24 ± 0.37
2	Phosphate buffer 4.5	99.34 ± 1.18	94.57 ± 1.23	89.04 ± 0.52

Observation

With phosphate buffer 6.8 and 4.5, the peak shape of Bepotastine Besilate was not symmetric in HPLC. Peak purity in HPTLC is retained.

CONCLUSION

Dissolution study of Bepotastine Besilate was monitored and compared by three analytical methods UV spectroscopy, HPLC, HPTLC for three different dissolution media. From the observations we concluded that all three analytical methods for Phosphate buffer 6.8 and 4.5 when compared with each other, found to have significantly different from each other. In case of 0.1 N HCl as dissolution media, HPLC and HPTLC showed degradation of Bepotastine Besilate in the dissolution media. Thus 0.1 N HCl is not suitable dissolution media for Bepotastine Besilate. UV spectrophotometry, being non-specific technique, was not able to detect change in 0.1 N HCl. Thus chromatographic technique is recommended for analysing dissolution samples.

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