Analytical Method Development and Validation for The Simultaneous Estimation of Bupro by Rp-HPLC Method

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ABSTRACT

A new procedure was established for simultaneous estimation of Bupro and Dopa by RP-HPLC method. The chromatographic conditions were successfully developed for the separations of Bupro by using Xterra C18.1 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosp buff (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophphoric acid), detection wave length was 255nm. The analytical method was validated according to I.C.H guidelines (I.C.H, Q.2 (R.1)).

KEY WORDS: Bupro R.P-H.P.L.C, validation.

1. INTRODUCTION

Analytical chemistry: Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behavior of matter.

Chromatography :The chromatograph was locate by Russaian Chemists and botanists Micheal Tswaett (1873-1918) who earliest uses the terms chromatographys to describing his works on the separations of coloureded plants pigments into bands on a columns of chalks and other materiasl such as polysaccharide, sucrose and insulins. “In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system.

“Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)”

1.2.3. Types of Chromatography : The mobile phases could be either a liquids or a gases, and accordinglly we can subdivide chromatography into Liquids Chromatography (LC) or Gases Chromatography (GC). Apart from these methods, there are two other modes that use a liquids mobile phases, but the nature of its transports throughs the porouses stationarys phase is in the form of eithers (a) capillary forces, as in planar chromatographyl (also called Thins-Layers Chromatographys, TLC), or (b) electros osmotics flowl, as in the case of Capillarys Electros Chromatographyl (CEC).

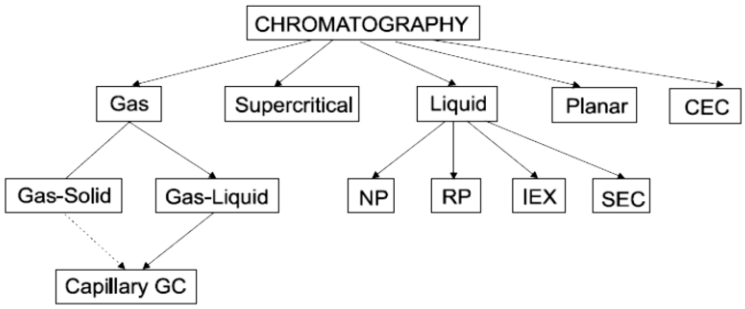


Figure 1. Showing flow chart for classification of chromatography

High Performance Liquid Chromatography (HPLC)

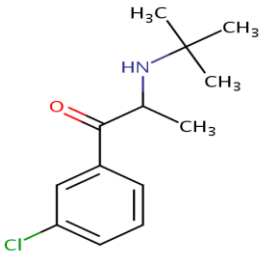
The acronym *HPLC*, coined by the Late Prof. Csabaa Horvaeth for his 1971 Pittconpapers, originally indicated the fact that high pressures was used to generates the flow requireds for liquids chromatographys in packeds columns.

DRUG PROFILE

BUPRO:

A unicyclic, aminoketone antidepressant. The mechanism of its therapeutic actions is not well understood, but it does appear to block Dopa uptake.

Molecular structure:



Chemical formula: $C_{13}H_{18}ClNO$
Molecular formula: 239.741

- **Categories:**
- Antidepressive Agents, Second-Generation
- Dopa Uptake Inhibitors.

LITERATURE REVIEW

1.Michael Elia El-Kommos et al., The method depends on the reaction of the cited proton pump inhibitors with diazotized p-nitroaniline in alkaline medium using 1.5 N NaOH and dimethylformamide. The

produced green color was measured at 590 nm for Bupro rabeprazole and 610 nm (for, pantoprazole and Dopa)

2. B.Santhoshas et al., A stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Bupro and Lafutidine in bulk and pharmaceutical dosage form. Chromatography carried on Thermo Hypersils BDS C18.1 (250mm x 4.6i.d, 5µm) column with mobile phase comprising of dipotass hydro phosp (0.1M) buff and meth in the ratio 60:40 v/v. The flow rate was adjusted to 1.0ml/min with UV detection at 280nm. The retention times of Domperidone, Lafutidine were found to be 1.813 min, 8.949 min respectively.

3. M. Sumithra et al., H.P.L.C Acme 9000 with UV/Vis detector with Inertsis ODS 3V, C18.1 (250×4.6×5µ) with an injection volume of 10µl is injected and eluted with the mobil phas of 0.03 M Ammon sulpha: Acetonit, pH-6.5 which is pumped at a flow rate of 1.6ml/min and detecteded by uv detectora at 250nm. The peaks of Pantopraz and Bupro are found well separated at 5.093 and 2.476 minutes respectively.

METHOD DEVELOPMENT:

Method development for simultaneous estimation of Bupro in Pharmaceutical dosage forms includes the following steps:

1. Selections of detections wavelengths (λ_{max})
2. Selections of columns
3. Selections of mobiles phasse
4. Selections of flow rates
5. Preparations and procedures

1. Selection of Detection wavelength: 10 mg of Bupro was dissolvings in mobile phase. The solutions were scanned from 200-400 nm the spectrum was obtained.

2. Selection of column: Column is selected based on solubilitys, polaritys and chemicals differences among Analytes [Column: Xterras C18.1 (4.5 x 230mm, 5µm, Make: Waters)]

3. Selection of mobile phase: Phost buff(0.05M) pH 4.6: ACN (55:45%v/v) has been selected as mobile phases. Buf pH should be between 2 to 8. If the buff pH is below 3 siloxane linkages are cleaved. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elutions properties by controlling the ionizations characteristics. It also decreases the retentions and improves separations. Good Response, Area, Tailing factosr, Resolutions will be achieved.

4. Selection of flow rate: Flow rate selected was 1ml/min Flow rate is selected based on

- Retention time
- Column back pressure
- Peak symmetry
- Separation of impurities

5. Preparations and procedures: ***Preparations of Phosphates buffer :(PH: 3.6):***

Weighed 6.8grams of KH2PO4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparations of mobile phases: A mixtures of pH 3.6 Phosphate buffer 550 mL (55%), 450 mL of ACN (45%) are taken and degassed in ultrasonic water bath for 5 minutes.

Procedure: 20µL of the standard, sample are injected into the chromatographic system and the areas for Bupro peaks are measured and the %Assay are calculated by using the formulae.

System Suitability: Tailing factors for the peaks due to **Bupro** in Standard solution should not be more than 2.0. and Bupro peaks in Standard solution should not be less than 2000

Assay calculation:

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where,
P = Percentage purity of working standard
Lc = LABEL CLAIM OF drug in mg/ml.

ANALYTICAL METHOD VALIDATION

ACCURACY:

Preparation of standard solution (Bupro): Accurately weighed 10mg of Bupro working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks.

Preparation of Sample solutions: For preparation of 50% solution (With respect to target Assay concentration): Accurately 5mg of Bupro working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

PRECISION

Repeatability: Preparation of standard stock solution: Accurately 10mg of Bupro working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Acceptance criteria: The % RSD for the area of five standard injections results should not be more than 2.

Intermediate Precision (Ruggedness):

To evaluate the intermediate precision (also known as ruggedness) of the Method, precision was performed on different days by using different make column of same dimensions.

Preparation of standard stock solution:

Accurately 10mg of Bupro working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Procedure

The standard solution was injected for five times and the area for all five injections measured in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The chromatograms

Acceptance criteria:The % RSD for the area of five sample injections results should not be more than 2%.

L.O.D:

L.O.D's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the L.O.D according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 3.3\ X\ \frac{\sigma}{S}$$

Where σ - Standard deviation (SD) S – Slope

L.O.Q:

L.O.Q's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

Formula:

$$\textbf{L.O.Q} = 10\,\sigma / \textbf{Slope}$$

Where

σ - Standard deviation

S – Slope

LINEARITY

Preparations of stocks solutions: Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Dopa and Bupro (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. .

Preparations of Levels – I (1pm of Bupro): 1ml of stocks solutions has taken in 10ml of volumetrics flasks and diluted up to the marks with diluents.

Preparations of Levels – II (2ppm of Bupro): 2ml of stocks solutions has taken in 10ml of volumetrics flasks and diluted up to the marks with diluents.

Preparations of Levels – III (3ppm of Bupro): 3ml of stocks solutions has taken in 10ml of volumetrics flasks and diluted up to the marks with diluent.

Preparations of Levels – IV (4ppm of Bupro): 4ml of stocks solutions has taken in 10ml of volumetric flasks and diluteds up to the marks with diluent.

Preparations of Levels – V (5ppm of Bupro) 5ml of stocks solutions has taken in 10ml of volumetric flasks and diluted up to the marks with diluents.

Procedure:

Each level was injected into the chromatographic system and the peak area was measured.

Acceptance criteria Correlation coefficient should be not less than 0.999.

RANGE:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Bupro and Dopa respectively.

ROBUSTNESS:

a) The flows rates was varied at 0.8ml/min to 1.2 ml/min. Standard solution 3ppm of Bupro was prepared and analyzed using the varied flow rates along with method flow rate.

SYSTEM SUITABILITY:

5 mg of Bupro working standard was accurateyy weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further 10 ml of Bupro was pipetted out from the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

RESULTS AND DISCUSSION:

WAVELENGTH DETECTION: The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. U.V range from 200-400nm. The overlay spectrum of Bupro was obtained and the isobestic point of Bupro and Dopa

showed absorbance's maxima at 255nm. The UV spectra of individual drugs are as follows:

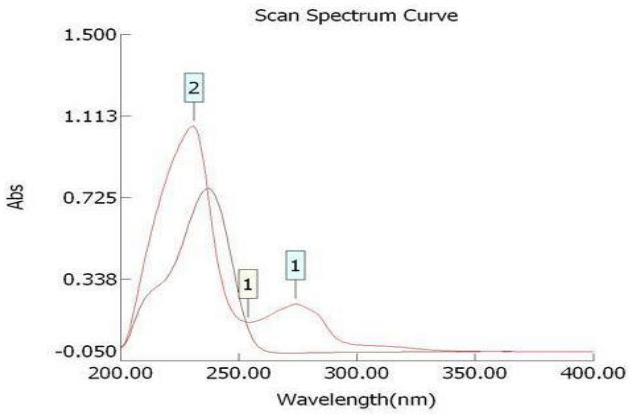


Figure-1: Overlay spectrum of Bupro

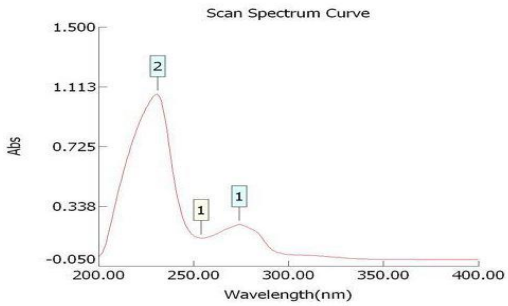


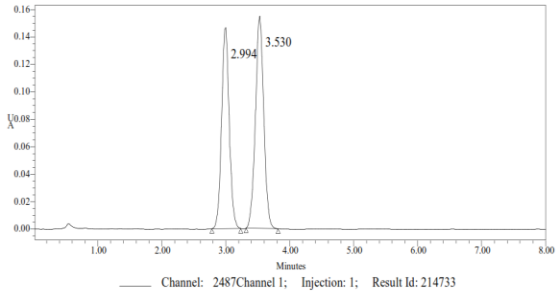
Figure-2: Spectrum of Bupro

METHOD DEVELOPMENT:

The chromatographic method development for the simultaneous estimation of Bupro and Dopa were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Bupro and Dopa in A.P.I and pharmaceutical dosage form by R.P-H.P.L.C method.

VALIDATION RESULTS

ACCURACY: The accuracy study was performed for 50%, 100% and 150 % for Bupro and Dopa. Each level was injected in triplicate into chromatographics system. The area of each level was used for calculation of % recovery.



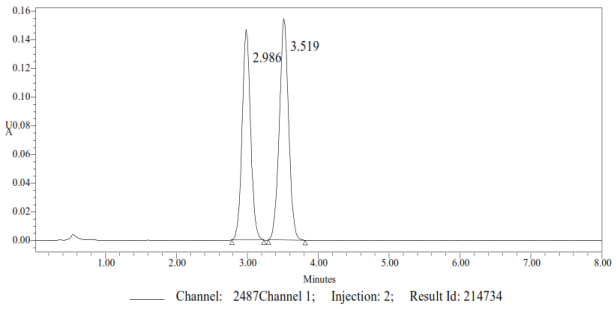


Figure-3 & 4: Chromatogram showing accuracy 50%injection-1 and injection-2

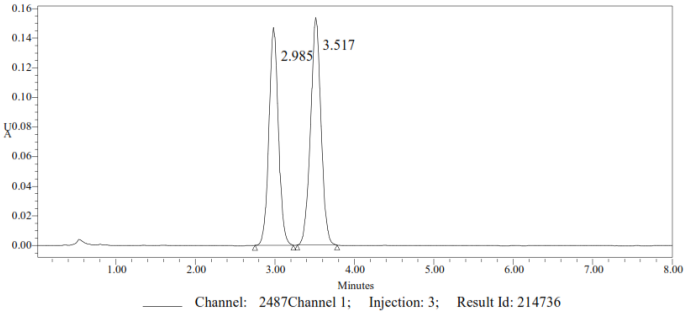


Figure-5: Chromatogram showing accuracy 50%injection-3

Table-1: Accuracy 50%

Name: Bupropion

	Name	RT	Area
1	**Bupropion**	3.530	641412
2	**Bupropion**	3.519	644644
3	**Bupropion**	3.517	648238
Mean			644765
Std. Dev.			3414.8
% RSD			0.52

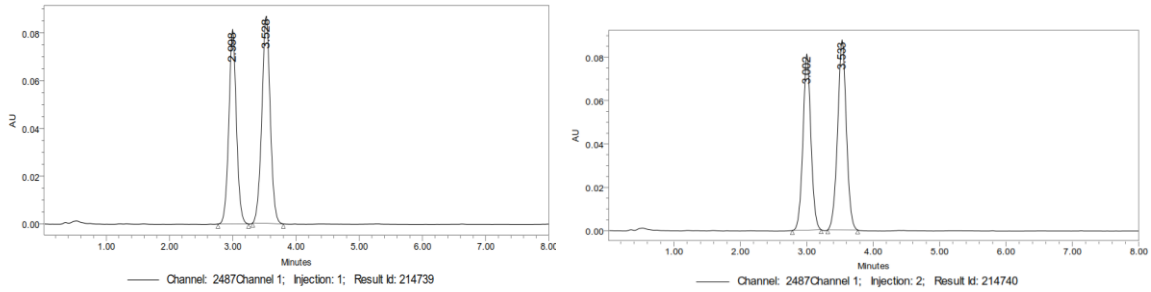


Figure-6 & 7: Chromatogram showing accuracy 100%injection-1 and Injection-2

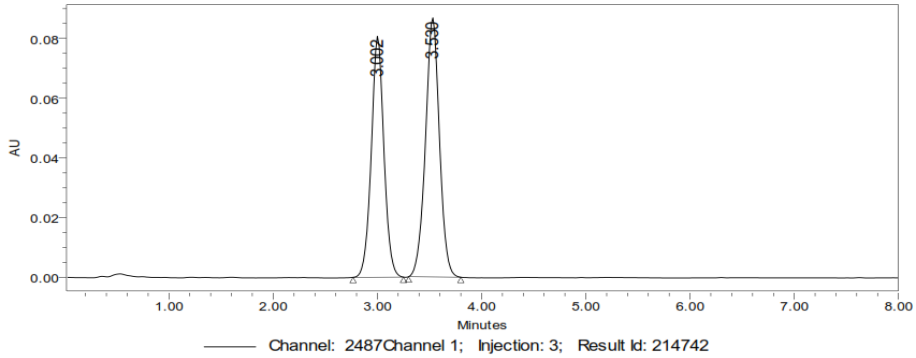


Figure-7: Chromatogram showing accuracy 100%injection-3

Table-2: Accuracy 100%

Name: Bupropion

	Name	RT	Area
1	**Bupropion**	3.528	798842
2	**Bupropion**	3.533	803075
3	**Bupropion**	3.530	809247
Mean			803722
Std. Dev.			5232.4
% RSD			0.65

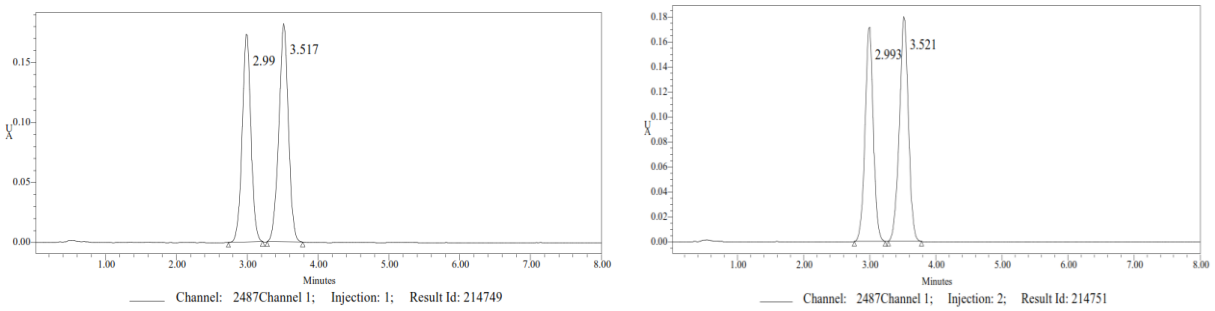


Figure-8 &9: Chromatogram showing accuracy 150%injection-1 and injection-2

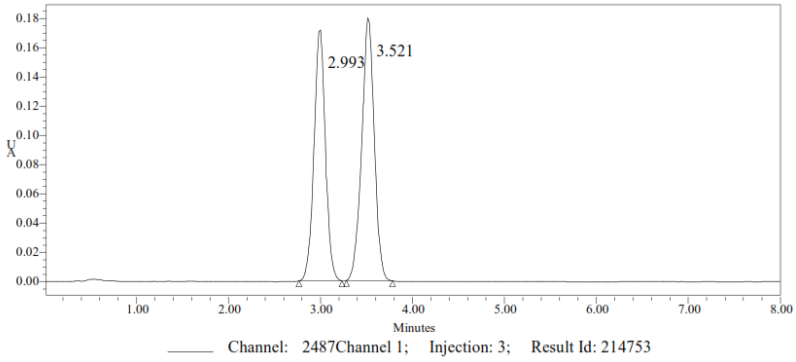


Figure-10: Chromatogram showing accuracy 150%injection-3

Table-3: Accuracy 150%

Name: Bupropion

	Name	RT	Area
1	**Bupropion**	3.517	960574
2	**Bupropion**	3.521	964089
3	**Bupropion**	3.521	964089
Mean			962917
% RSD			0.2

The accuracy results for Bupro :
Table-11: Accuracy results of Bupro

%Concentration (at specification level)	Area	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	644765	5	5.0	101.3%	100.0%
100%	803722	10	9.94	99.4%	
150%	962917	15	14.8	99.2%	

AcceptaCriteria;
The % Recovery for each level should be between 98.0 to 102.0%

PRECISION
i) Repeatability
ii) Intermediate precision (Ruggedness)
Repeatability

The precision study was performed for five injections of **Bupro** and **Dopa**. Each standard injection was injected in to chromatographic systems. The area of each Standard injection was used for calculation of % RSD.

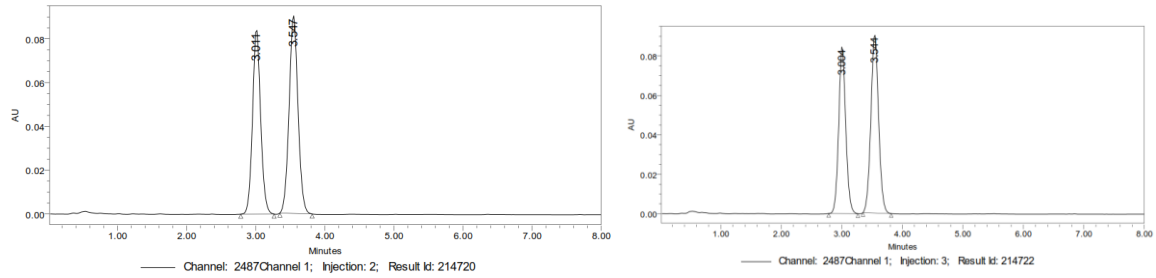


Figure-11& 12: Chromatogram of Standard Inj-1 and Inj-2

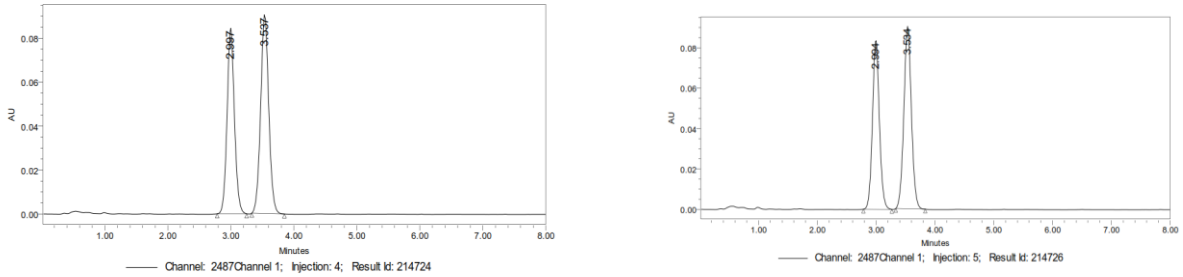


Figure-13 & 14: Chromatogram of Standard Inj-3 and Inj-4

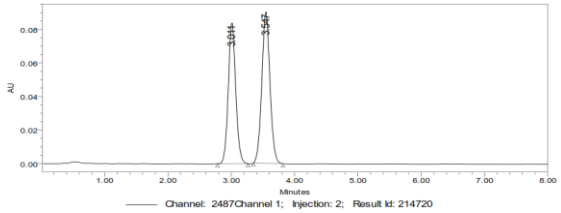


Figure-15: Chromatogram of Standard Inj-5

Table-12: Repeatability results of Bupro

Name: Bupropion

	Name	RT	Area
1	**Bupropion**	3.557	819305
2	**Bupropion**	3.547	807157
3	**Bupropion**	3.544	804070
4	**Bupropion**	3.537	808474
5	**Bupropion**	3.534	804505
Mean			808702
Std. Dev.			6203.7
% RSD			0.77

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2% .The Method precision study was performed for the %RSD of Bupro and Diazepam was found to be 0.7 and 0.4 (NMT 2).

Intermediate precision/Ruggedness: The intermediate precisions study was performed for five injections of Bupro and Dopa. Each standard injection was injected into chromatographic systems. The area of each standard injections was used for calculation of % RSD.

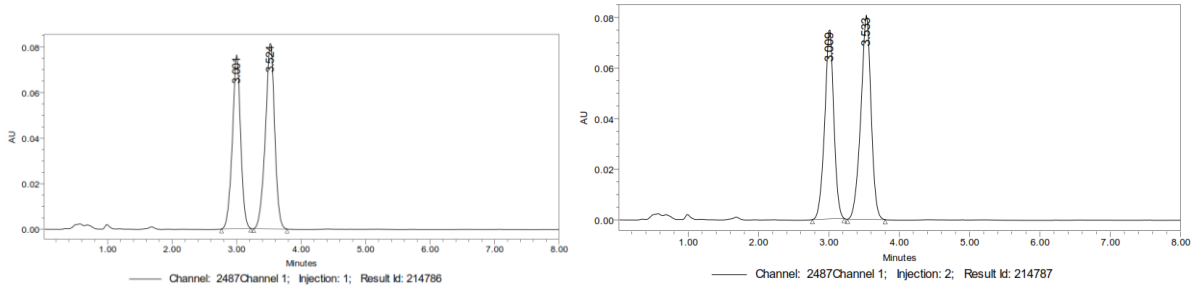


Figure-16 &17: Chromatogram of Standard Inj-1 and Inj-2(ID Precision)

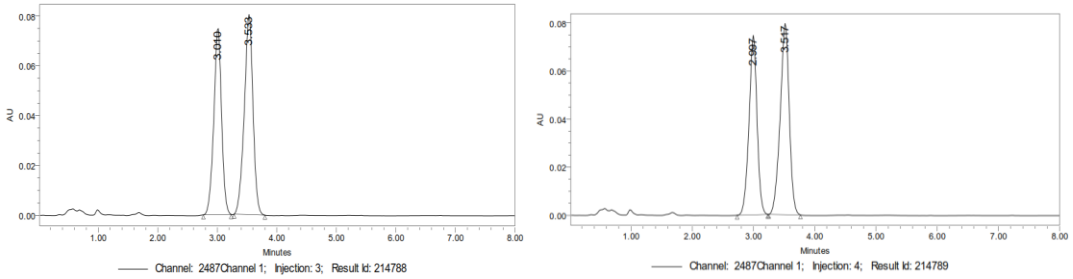


Figure 18& 19: Chromatogram of Standard Inj-3 and Inj-4(ID Precision)

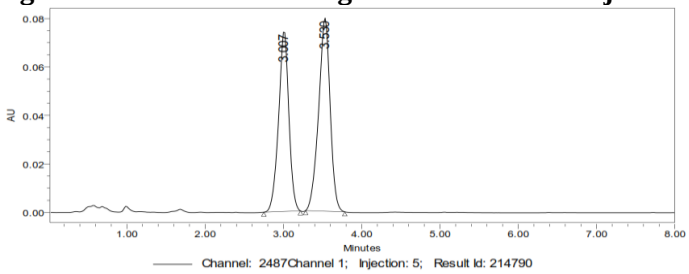


Figure-20: Chromatogram of Standard Inj-5(ID Precision)

Table-13: Ruggedness results of Dopa

Name: Bupropion

	Name	RT	Area
1	**Bupropion**	3.524	813507
2	**Bupropion**	3.533	817673
3	**Bupropion**	3.533	815189
4	**Bupropion**	3.517	815816
5	**Bupropion**	3.530	815356
Mean			815508
Std. Dev.			1492.7
% RSD			0.18

Acceptance Criteria: The % RSD for the area of five standards injections results should not be more than 2%. The intermediate precisions was performed for %RSD of Bupro and Dopa was found to be 0.18 and 0.39 respectively (NMT 2).

SPECIFICITY:

The system suitability for specificity was carried out to determines whether there is any interference of any impurities in retention time of analytical peak.

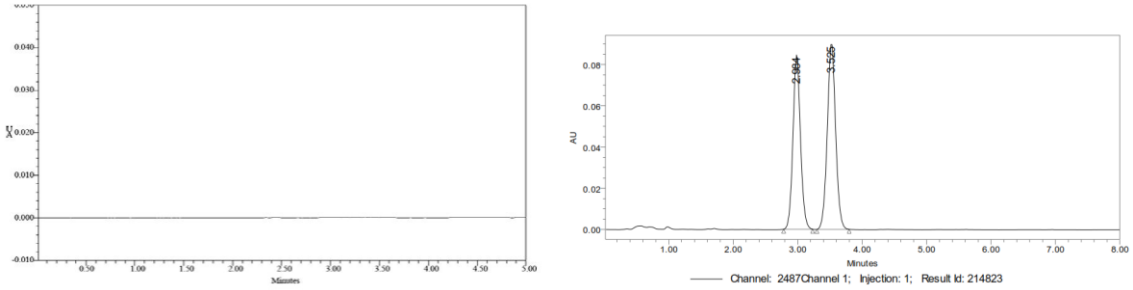


Figure 21 & 22: Chromatogram of blank Injection and Standard Injection

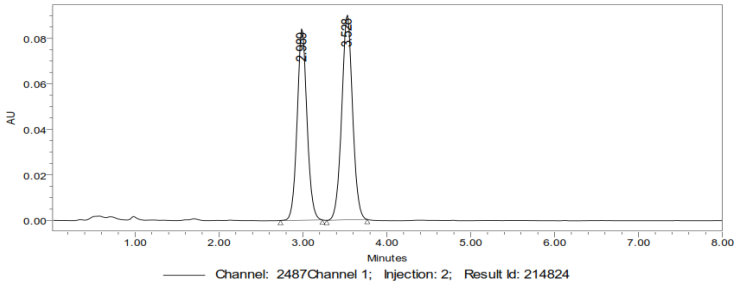


Figure-23: Chromatogram of Standard Injection

Table-14: Details of Standard Injection

Name: Bupropion

	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	**Bupropion**	3.525	810802	3527.8	1.0	2.4
2	**Bupropion**	3.528	808790	3566.2	1.0	2.3
Mean			809796	3547.0	1.0	
Std. Dev.			1422.2			
% RSD			0.18			

The specificity test was performed for Bupro. It was found that there was no interference of impurities in retention time of analytical peaks.

DETECTION OF LIMIT: L.O.D's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the L.O.D according to the formula. The standards deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\mathbf{LOD} = 3.3 \, \mathbf{X} \, \frac{\sigma}{S}$$

Where
σ - Standard deviations (SD)
S – Slope

Bupro

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 41 µV Signal
Obtained from L.O.D solution : 121 µV
S/N = 121/41 = 2.95

Acceptance Criteria:

S/N Ratio value shall be 3 for L.O.D solution.
The L.O.D was performed for Bupro and Dopa was found to be 2.95and 3.04 respectively.

QUANTITATION LIMIT:

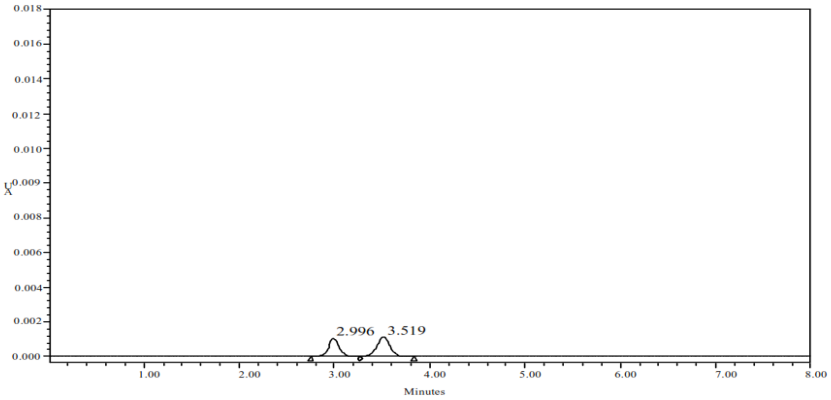


Figure-24: Chromatogram of L.O.Q

Bupro

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 41 µV Signal
Obtained from L.O.Q solution : 405µV
S/N = 405/41 = 9.87

Acceptance criteria

S/N Ratio value shall be 10 for L.O.Q solution. The L.O.Q was performed for Bupro and Dopa was found to be 9.87and 10 respectively**.**

SUMMARY AND CONCLUSION:

A recently technique was established for simultaneous estimation of Bupro by R.P-H.P.L.C method. The chromatographic conditions were successfully developed for the separations of Bupro by using Xterras C18.1 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phases ratio was Phosphate buf (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthopho acid), detections wave lengths was 255nm.The retention times were found to be 2.984mins and 3.525mins. The % purity of Bupro was found to be 100.7% and 101.4% respectively.

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