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Analytical method development of Bromacil + Terbuthylazine pesticide (combination) formulation by Reverse Phase High-Performance Liquid Chromatography (R–HPLC)

Ayyavoo Kaliyan
ayyavoo_vasanth@yahoo.com
Bioscience Research Foundation, Kandamangalam, Tamil Nadu

Dr. C. Tamilselvan
brfchennai@gmail.com
Bioscience Research Foundation, Kandamangalam, Tamil Nadu

ABSTRACT

The combination of the two molecules (Bromacil & Terbuthylazine) is being used effectively as a weedicide worldwide due to its unique way of acting to kill the weeds. Since these two molecules (Bromacil & Terbuthylazine) has very good penetrating power in the earth crust, it is very important to get analysed for its identification and quantification to determine the shelf life in the substrate by detecting the minimum residue level (MRL) in the existing substrate. A simple HPLC chromatographic method has been developed and subsequently validated for the combination pesticide (Bromacil + Terbuthylazine) separation and quantification. These molecules were separated through a mobile phase consisting of the mixture of acetonitrile and water ratio of 80:20 v/v. The separation was achieved through the Qualisil BDS C18 (250 x 4, 5µ) column with the flow rate as 1.0 ml/min with the detection at 220 nm. These method parameters were loaded in the Shimadzu HPLC (model: LC-2030). The LC solution Shimadzu software was used for all the calculations in this analytical method validation analysis. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the identification and quantifications of these molecules interims of validation parameters viz., separation, system suitability, System Precision and linearity in a simple HPLC analysis.

Keywords— *Bromacil + Terbuthylazine, HPLC analysis, Validated method, SANCO 3030/99 Rev.4, ICH guideline*

1. INTRODUCTION

The formulations development processes is a continuous process in many areas, in the pesticide industry also it is a very fast development with more than one pesticide formulations. The requirements of this kind of formulations being decided based on the need of the applications for multi-tasking. Bromacil is being used as chemical as well as a weedicide in the scientific world. Bromacil molecule is a form of derivative of Uracil molecule. In the Uracil molecule, the acidic protons of the molecule were replaced with alkane and bromide. Over all the Bromacil have many functional groups, viz., bromide, secondary amine, ketone and the tertiary amine in its molecular structure. This Bromacil molecule has the power of penetrating through all the surface of the Earth crust like water sand, mud, and event through the plant roots. To destroy the weeds or unwanted plants the Bromacil molecule applied effectively since 1960. The penetrating power of the molecule might have derived due to the presence of the bromide and amine groups existing in the molecular structure. The Terbuthylazine molecule is an organic hetero cyclic chemical derivative of triazine with chloride and substituted amine groups. This Terbuthylazine molecule applied as a weed killer since time by the farmers. This molecule also has the power of penetration through the roots and surface of leafs of the plants and weeds. This molecule also breakdown the photosynthesis of the leaf and hence the weeds being controlled selectively. The leaf and roots entering a mode of action of this molecule are one of the fastest ways of controlling weeds selectively.

This effectively used weedicide has to be analysed to determine the next usage of the substrate effectively. Therefore it is important to understand the active content of these molecules with a single analysis.

2. MATERIALS AND METHOD

2.1 Reagents and chemicals used: All the analytical grade solvents and water were used in this analytical method development. A class A grade glass was used in this research analytical method development.

2.2 Instrument: A calibrated chromatography HPLC instrument was used to develop this analytical method development for Terbuthylazine and Bromacil. The instrument parameters were given as:

Name of the instrument	:	High Performance Liquid Chromatography (HPLC)
Calibration Method	:	External Standard Method
Make	:	Shimadzu
Model	:	LC 2030
Detector	:	UV-Visible
Wavelength (λ)	:	220 nm
Column Temperature	:	30ºC
Column	:	Qualisil BDS C18 (250 x 4.6 mm, 5µ)
Mobile Phase	:	Acetonitrile: Water; 80:20 (v/v)
Flow rate	:	1.0 ml/min
Injection volume	:	10 µl
Retention time (Approximately)	:	Terbuthylazine– 5.3 minutes
	:	Bromacil– 3.6 minutes
Total Run time	:	10 min.

2.3 Preparation of Mobile phase

A volume of 80% Acetonitrile and 20% of HPLC grade water mixed well, sonicated and used for analysis.

3. ANALYTICAL METHOD VALIDATION

3.1 Specificity

3.1.1 Preparation of standard stock solutions: An amount of 10.05 mg of Terbuthylazine reference standard with purity 99.5% and 10.15 mg of Bromacil reference standard with purity 98.5 % was weighed accurately into a clean and dry 10 mL volumetric flask separately and dissolved in mobile phase and made up to the mark with the mobile phase. This was equivalent to each 1000 mg/L; from this, each 2.5ml solution was added in 25 ml volumetric flak and diluted with mobile phase. This solution was equivalent to 100 mg/L and analyzed to determine specificity.

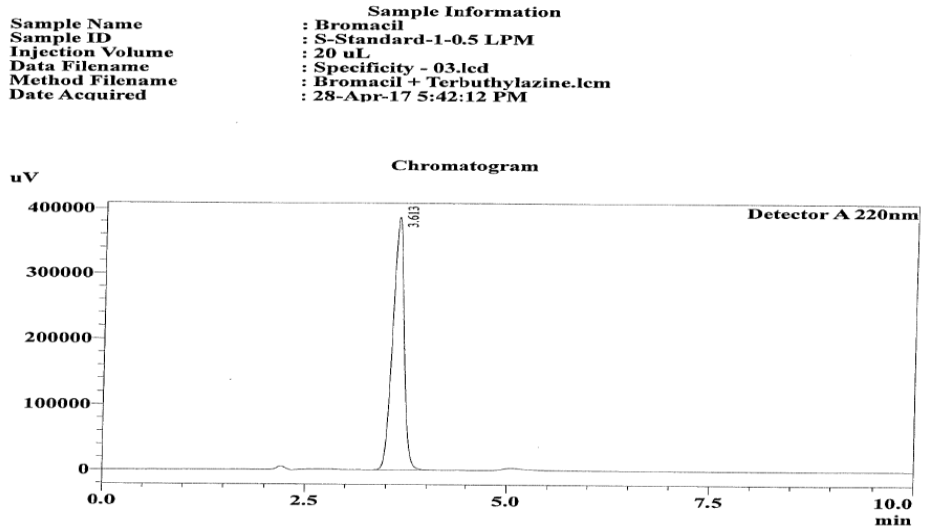

Fig. 1: A typical Chromatogram for specificity (Bromacil)

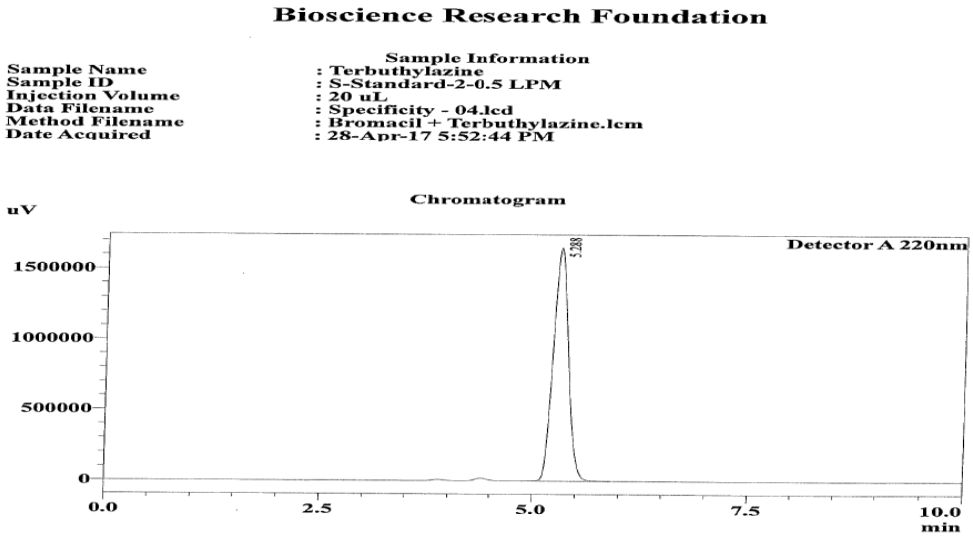

Fig. 2: A typical Chromatogram for Specificity (Terbuthylazine)

3.1.2 Preparation of Sample Solution

An amount of 10.0 mg of the test substance was weighed accurately into a clean and dry 100 mL volumetric flask and dissolved in mobile phase and made up to the mark with the mobile phase. This was equivalent to 100 mg/L. This prepared solution was used for the determination of Specificity.

The specificity of HPLC method for Terbuthylazine 16% and Bromacil 4% SC was determined by injecting the Standard and Sample solutions along with blank (mobile phase) and observed that there was no interference found with the main peak of interest. Hence, this method was considered to be specific for the analysis of Terbuthylazine and Bromacil.

3.2 Linearity

3.2.1 Preparation of Standard Stock Solution and working standard: An amount of 10.0 mg of each (Terbuthylazine and Bromacil) standard was weighed into a 100 ml standard flask and this concentration (100 mg/L) was used to prepare further dilutions to get the 10, 20, 30, 40, 50 and 60 mg/L separately. The dilution details are presented in table No.1

Table 1: Dilutions (Bromacil 80 + Terbuthylazine reference standard)

Standard Code	Stock Dose (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
1	100	1	10	10
2	100	2	10	20
3	100	3	10	30
4	100	4	10	40
5	100	5	10	50
6	100	6	10	60

The prepared standard solutions were injected by an auto sampler into the HPLC system and a linear curve was plotted for the concentration of standard versus observed peak area and the correlation coefficient was determined respectively. The results are presented in table 2 and 3.

Table 2: Linearity of bromacil reference standard

Std. level	Concentration (ppm)	Reputability	Area Response	Mean Area
Std Conc.-1	10	R1	340805	341022
		R2	341239	
Std Conc.-2	20	R1	679563	679558.5
		R2	679554	
Std Conc.-3	30	R1	1016509	1018706
		R2	1020903	
Std Conc.-4	40	R1	1385265	1384949
		R2	1384633	
Std Conc.-5	50	R1	1709966	1713829
		R2	1717692	
Std Conc.-6	60	R1	2092627	2093223
		R2	2093819	
			Intercept	-17791.36667
			Slope	34943.02714
			Correlation Coefficient	0.999835279

Table 3: Linearity of Terbuthylazine reference standard

Std. level	Concentration (ppm)	Reputability	Area Response	Mean Area
Std Conc.-1	10	R1	1863100	1862833
		R2	1862566	
Std Conc.-2	20	R1	3597783	3597576
		R2	3597369	
Std Conc.-3	30	R1	5364096	5366226
		R2	5368356	
Std Conc.-4	40	R1	7266319	7268394
		R2	7270469	
Std Conc.-5	50	R1	9176082	9169694
		R2	9163306	
Std Conc.-6	60	R1	10910274	10909970.5
		R2	10909667	
			Intercept	-22972.03333
			Slope	182440.5986
			Correlation Coefficient	0.999855488

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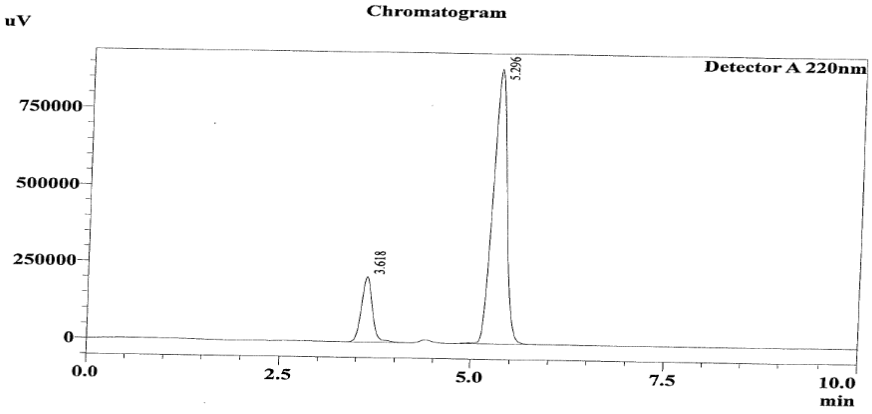
Sample Information

Sample Name	: Bromacil + terbuthylazine
Sample ID	: L-Std-6-R1-0.5 LPM
Injection Volume	: 20 uL
Data Filename	: Linearity - 12.lcd
Method Filename	: Bromacil + Terbuthylazine.lcm
Date Acquired	: 28-Apr-17 8:20:00 PM



Peak Table

Detector A 220nm

Peak#	Ret. Time	Area	Area%	Height	Name
1	3.618	2092627	16.094	211475	Bromacil
2	5.296	10910274	83.906	890415	Terbuthylazine
Total		13002901	100.000	1101890	

Fig. 3: A typical Chromatogram for Linearity 60 mg/L (R1)

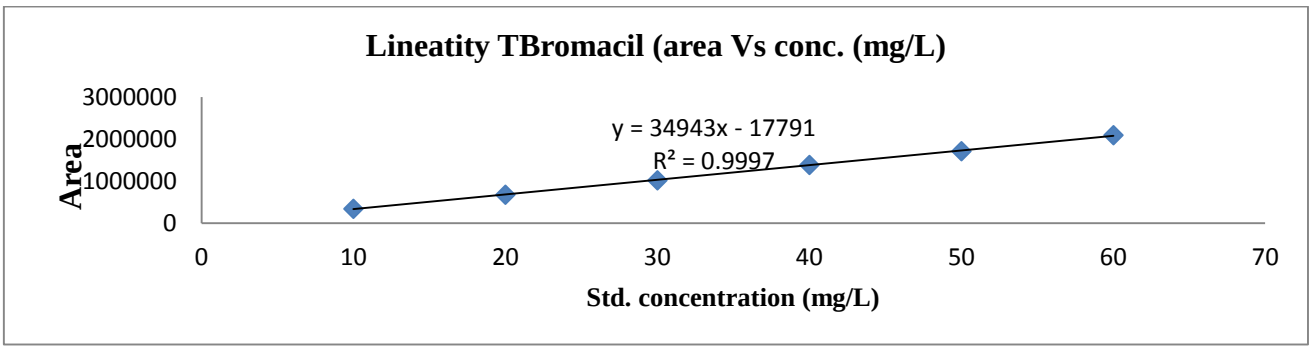


Fig. 4: Linearity curve for Bromacil

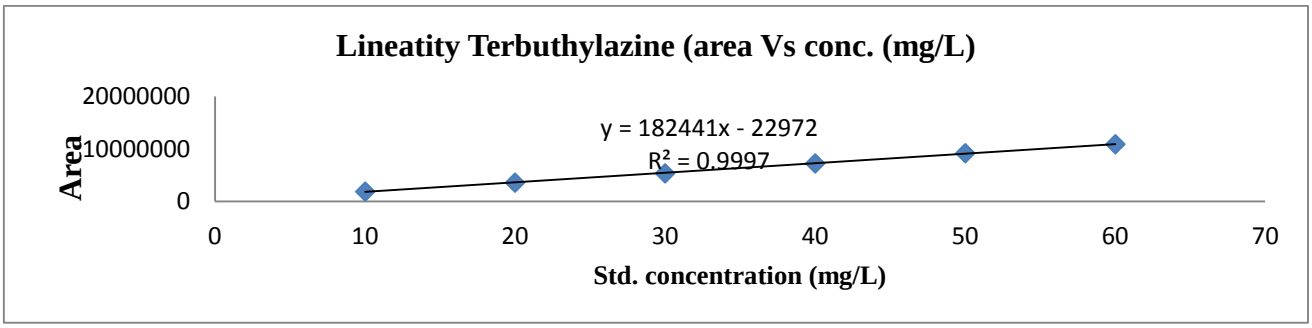


Fig. 5: Linearity curve for Terbuthylazine

4. PRECISION

4.1 Preparation of Standard Solution

The Linearity standard solution 30 mg/L was prepared and used for the precision determination.

4.2 Preparation of Sample Solution

An amount of 30 mg of Bromacil 80 + Terbuthylazine Technical was weighed in clean and dry 1000 ml volumetric flask separately, dissolved the contents with mobile phase and made up to the mark with the mobile phase. These solutions are equivalent to 30 mg/L. The prepared solutions were injected into HPLC and % RSD was calculated and the results are presented in Table 4.

Table 4: Precision (Bromacil)

Code	Standard (S)/Sample (w) concentration (mg/L)	Standard Area (Hs) / Sample Area (Hw)	Standard Average Area	Purity (P) %	A.I Content (% w/w)
STD - R1	30	1018349	1018780	-	-
P1	30	81998		98.50	7.93
P2		82204			7.95
P3		82257			7.95
P4		81990			7.93
P5		82184			7.95
STD - R2	30	1019211		-	-
				MEAN	7.94
				SD	0.012
				RSD	0.15

Table 5: Precision (Terbuthylazine)

Code	Standard (S)/Sample (w) concentration (mg/L)	Standard Area (Hs) / Sample Area (Hw)	Standard Average Area	Purity (P) %	A.I Content (% w/w)
STD - R1	30	5375624	5378466	-	-
P1	30	656520		99.50	12.15
P2		655112			12.12
P3		655190			12.12
P4		658415			12.18
P5		658225			12.18
STD - R2	30	5381308		-	-
				MEAN	12.15
				SD	0.029
				RSD	0.24

Formula:

$$\text{A. I. Content (\%)} = \frac{\text{Sample Area x Std. Conc. (mg/L)}}{\text{Average Std. Area x Sample Conc. (mg/L)}} \text{x Purity (P) \%}$$

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.413 RSD for 100% active analyte as per SANCO/3030/99 Rev.4)

5. ACCURACY (% RECOVERY)

The recovery processes and the recovery determination was validated with two fortification level of processes.

5.1 Preparation of Standard Solution

The standard solution prepared 25 mg/L was prepared from the stock standard solution 1000 mg/L was used as a standard in percent recovery determination.

5.2 Preparation of Fortification Level 1 (35 mg/L)

An aliquot of 3.5 mL of above standard stock solution (100 mg/L) was transferred into a 10 mL volumetric flask and fortified in distilled water, sonicated and made up to the mark with the distilled water. This solution was equivalent to 35 mg/L.

5.3 Preparation of Fortification Level 2 (50 mg/L)

An aliquot of 5.0 mL of above standard stock solution (100 mg/L) was transferred into a 10 mL volumetric flask and fortified in distilled water, sonicated and made up to the mark with the distilled water. This solution was equivalent to 50 mg/L.

The above preparations were analyzed under HPLC and checked for recovery (%). The results are presented in following table 6 and 7

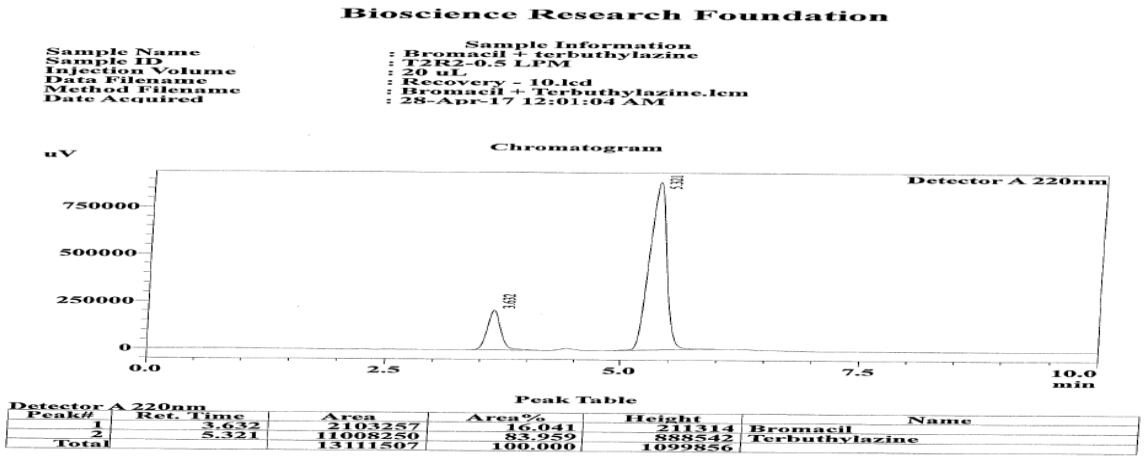


Fig. 6: A typical Chromatogram for Recovery (Bromacil + Terbuthylazine)

Table 6: Accuracy (Level-1 and 2 Recovery %) Of Bromacil

Fortification Level	Std. Conc. (mg/L)	Std. / Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)
Std-R1	25	1019326	1019427	-	35	-	-
T1R1		1391944		34.1355		97.53	97.53
T1R2		1391774		34.1313		97.52	
T1R3		1392503		34.1492		97.57	
T1R4		1391619		34.1275		97.51	
T1R5		1391719		34.1299		97.51	
T2R1		2102402		51.5584	50	103.12	103.14
T2R2		2103257		51.5794		103.16	
T2R3		2102780		51.5677		103.14	
T2R4		2103367		51.5821		103.16	
T2R5		2102765		51.5673		103.13	
Std - R2		1019528		-		-	

Table 7: Accuracy (Level-1 & 2 Recovery %) OF Terbuthylazine

Fortification Level	Std. Conc. (mg/L)	Std. / Sample area	Mean Std. Area	Recovery Conc. (mg/L)	Fortified Conc. (mg/L)	Recovery (%)	Avg. Recovery (%)
Std-R1	25	5381972	5383668	-	35	-	-
T1R1		7302801		33.9118		96.89	97.30
T1R2		7303351		33.9144		96.90	
T1R3		7361653		34.1851		97.67	
T1R4		7353044		34.1451		97.56	
T1R5		7347936		34.1214		97.49	
T2R1		11014040		51.1456	50	102.29	102.02
T2R2		11008250		51.1187		102.24	
T2R3		10963924		50.9129		101.83	
T2R4		10966940		50.9269		101.85	
T2R5		10969819		50.9403		101.88	
Std - R2		5385364		-		-	

5.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

From the Linearity Standard Solution concentration of 30 mg/L was used to prepare 1 mg/L standard mixture solution; From this solution, 1 mg/L solution was prepared and further diluted to get the 0.2 & 0.1 mg/L concentration solutions were prepared. The dilution details were given in Table 8, and the results are presented in following Table 9 and table 10.

Table 8: Dilutions (LOD & LOQ)

Stock concentration (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
1.0	0.2	10	0.02
1.0	1.0	10	0.1

Formula:

LOD = Average + (3 x Standard Deviation)
LOQ = Average + (10 x Standard Deviation)

Table 9: Limit of Detection (LOD) and Limit of Quantification (Loq) of Bromacil

Std. Code	Std. Area	Std. Conc. (mg/L)	Mean std. area	LOD (mg/L)
Std-R1	1019954	30	1020228	-
LOD-R1	2820	-		0.083
LOD-R2	2288			0.067
LOD-R3	2241			0.066
Std-R2	1020502	30		-
			MEAN	0.072
			SD	0.01
			LOD	0.10

Std. Code	Std. Area	Std. Conc. (mg/L)	Mean std. area	LOD (mg/L)
Std-R1	1019954	30	1020228	-
LOD-R1	7178	-		0.211
LOD-R2	7171			0.211
LOD-R3	7071			0.208
Std-R2	1020502	30		-
			MEAN	0.210
			SD	0.002
			LOQ	0.22

Table 10: limit of Detection (LOD) and Limit of Quantification (LOQ) OF Terbuthylazine

Std. Code	Std. Area	Std. Conc. (mg/L)	Mean std area	LOD (mg/L)
Std-R1	5387939	30		-
LOD-R1	16302			0.09075
LOD-R2	16337	-	5388958	0.09095
LOD-R3	16293			0.09070
Std-R2	5389977	30		-
			MEAN	0.091
			SD	0.0001
			LOD	0.09

Std. Code	Std. Area	Std. Conc. (mg/L)	Mean std area	LOD (mg/L)
Std-R1	5387939	30		-
LOD-R1	68885			0.38348
LOD-R2	67940	-	5388958	0.37822
LOD-R3	68547			0.38160
Std-R2	5389977	30		-
			MEAN	0.38
			SD	0.0027
			LOQ	0.389

6. LOD & LOQ Formula

LOD = Average + (3 x Standard Deviation).
LOQ = Average + (10 x Standard Deviation)

6.1 Limit of Detection

$$\text{A. I Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc. (mg/L)} \times \text{Sample Area}}{\text{Average Std. Area}}$$

LOD = Mean Value + (3 × SD)

6.2 Limit of Quantification

$$\text{A. I Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc. (mg/L)} \times \text{Sample Area}}{\text{Average Std. Area}}$$

LOQ = Mean Value + (10 × SD)

7. ACTIVE CONTENT ANALYSIS OF BROMACIL + TERBUTHYLAZINE

7.1 Preparation of Standard solution

An amount of 10 mg of the standard was dissolved in 100 ml of mobile phase and diluted to get 100 mg/L was used as a standard in concentration analysis.

7.2 Preparation of Sample Solutions

The test solutions (30 mg/mL) was prepared and dissolved by sonication and diluted appropriately and injected into HPLC.

$$\text{Bromacil 80 + Terbuthylazine } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{A \times B \times DF}{C}$$

Where,

A - Concentration of standard (ppm)
B - Area of the sample solution
C - Area of standard solution
DF - Dilution Factor

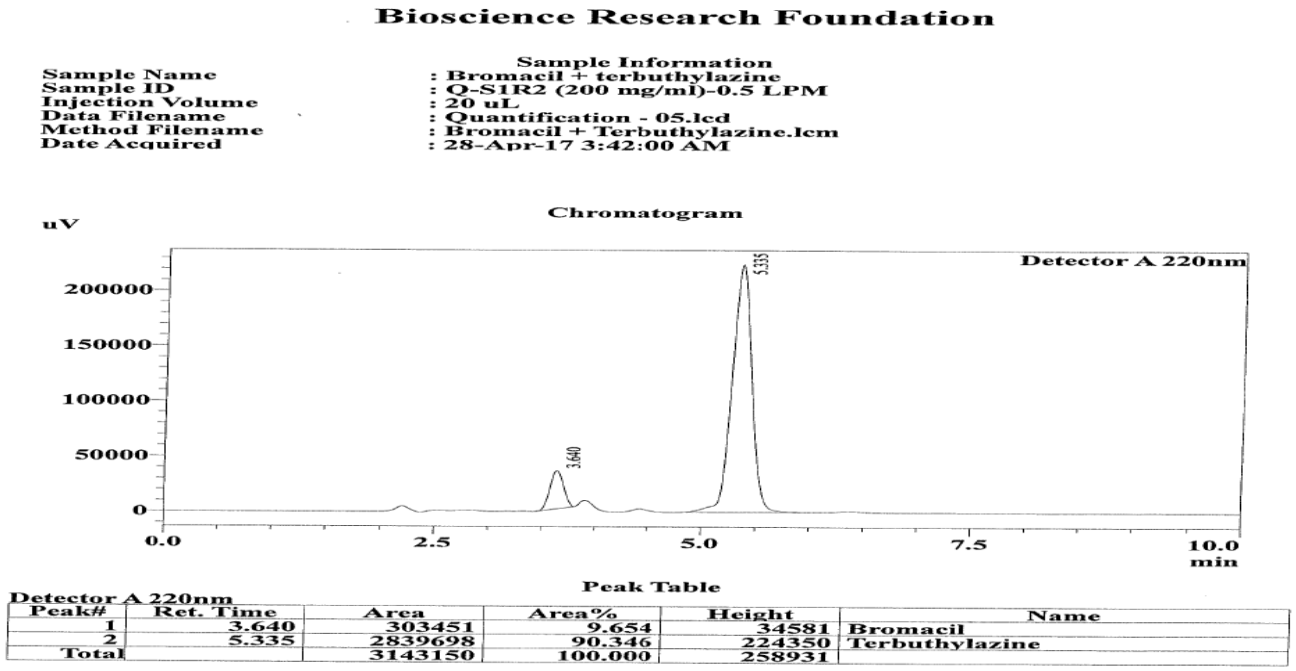


Fig. 7: A Typical Chromatogram for Sample analysis

8. CONCLUSION

8.1 Specificity: The blank, standard and the sample peaks did not interfere with each other, hence the specificity was achieved as per the guideline SANCO 3030/99 Rev.4 requirement.

8.2 Linearity: The Linearity correlation co-efficient is achieved NLT 0.99 as per (SANCO 3030/99 Rev.4

8.3 System Precision: The system precision is achieved as the % RDS for 5 replicates observed as 0.1% for Bromacil 80g/Kg + Terbuthylazine 120g/Kg, hence the minimum requirement of the (SANCO 3030/99 Rev.4 was NMT 15% RSD was achieved

8.4 System Recovery: The system recovery 92% to 101 % were achieved for Bromacil 80g/Kg + Terbuthylazine 120g/Kg, hence the minimum requirement of the (SANCO 3030/99 Rev.4).

8.5 System Suitability: The HPLC method is suitable for analysis of the combination product of Bromacil 80g/KG + Terbuthylazine 120g/Kg to detect up to 0.01 µg/g in the formulation state.

Details of the Laboratory work were carried out

Bioscience Research Foundation, Sengadu village & Post, Via Manavalanagar, Kandamangalam – 602002, Kanchipuram District, Tamil Nadu, India Ph: +91 44 27601082, Email: brfchennai@gmail.com

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