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Separation and quantification of Chloro Triazine based Atrazine and Terbuthylazine formulation by reverse phase high performance liquid chromatography

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ABSTRACT

The Atrazine and Terbuthylazine molecules are being used alone and a combination as a special herbicide activity to control the broadband leaves in the agricultural industry. Atrazine and Terbuthylazine have a high capillary action in the roots of brad spectrum plants with respect to other herbicide molecules. The solubility of Atrazine and Terbuthylazine also very high solubility in water and this solubility enhanced the capillary action through the broad leaves plant roots. The persistence of this Atrazine and Terbuthylazine is very long period hence the residue levels in the used substrates are being existed in the used substrates. Even though the molecules Atrazine and Terbuthylazine are less toxic to humans and animals, the lowest detection levels have to be determined with a simple HPLC analytical method; which is very effective time and cost of analysis. Within 10 minutes of analysis time these two molecules to be determined by using acetonitrile and water as a mobile phase with a ratio of 80:20 (volume/volume) with the help of Quails BDS C18 (250 x 4.6, 5µ) HPLC column at 1 ml/min. flow rate. The detection wavelength is 220 nm by a Shimadzu LC2030 model HPLC. The results of the analysis deliver 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— *Atrazine and Terbuthylazine, HPLC analysis, Validated method, SANCO 3030/99 Rev.4, ICH guideline*

1. INTRODUCTION

Atrazine is one of the selective herbicide, which contains the chloro triazine chemical unit. This unit has two secondary amines with isopropyl and ethyl attached system separately. This molecule has high penetrating power in all the substrate available on the earth crest. This high penetrating power is one of the physical properties of Atrazine molecule. Moreover, this atrazine molecule has high boiling and melting points which help to forbidden the vaporization of this molecule; this resulted to retain in many of the substrate high in levels. The soil, water and treated plant parts retain the atrazine molecule as residue due to this physical nature the sample molecule containing another selective herbicide is Terbuthylazine have tertiary butyl attachment in the secondary amine molecule and the only different structural arrangement differs from Atrazine. Hence this two molecule combination working as a very effective herbicide and also retains on the earth crust more in quantity. Hence this two herbicide combination being used as a very good effective herbicide and also retains on the Earth crest more in quantity. Analysis of these two herbicides is most important in terms of identification and quantifications. Since the retaining in the substrate rate is very high and the analytical technique also to be very precious to identify and quantify with minimum time and cost.

2. MATERIALS AND METHOD

2.1 Reagents and chemicals used

All the analytical grade solvents and water were used in this analytical method development. All the class a glass wear used in this research analytical method development.

2.2 Instrument

In this experiment used HPLC was periodically calibrated and maintained to develop this analytical method development for chloro triazine compounds (Atrazine and Terbuthylazine). The HPLC make Shimadzu, Model LC 2030; Detector UV-Vis.; Absorption at 220 nm; Column used, Qualisil BDS C18 (250 x 4.6, 5µ); mobile phase used Acetonitrile and Water; the ratio of 80:20 (v/v) with flow rate 1 ml/min. With this HPLC condition, the chlorotriazine molecules Atrazine and Terbuthylazine were eluted at 3.4 minutes and 4.0 minutes respectively.

2.3 Preparation of Mobile phase

A volume of 80% Acetonitrile and 20% were 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 10.09 mg Atrazine reference standard with purity 99.1% and 10.05 mg Terbuthylazine reference standard with purity 99.5% were weighed accurately into a clean and dry 10 mL volumetric flask separately, dissolved with mobile phase and made up to the mark with the mobile phase. This solution was equivalent to 1000 mg/L respectively. From this, an aliquot of each 1ml solution was mixed 10 mL volumetric flask, diluted with the mobile phase. This solution was equivalent to 100 mg/L and analyzed to determine specificity.

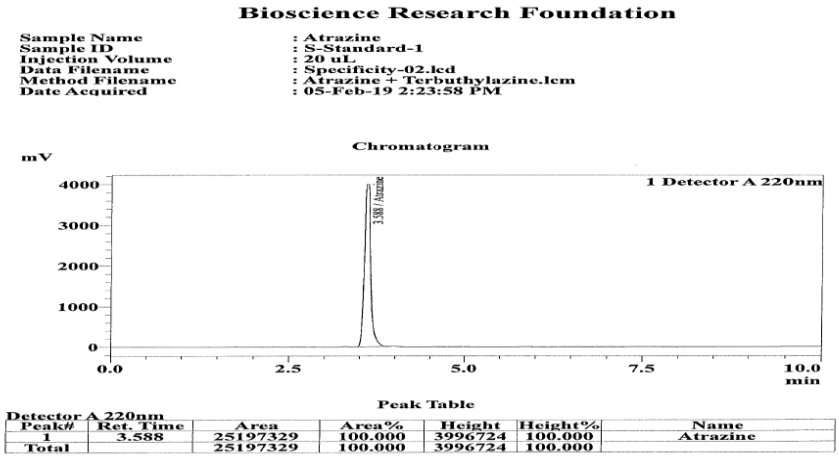

Fig. 1: Typical Chromatogram for Atrazine

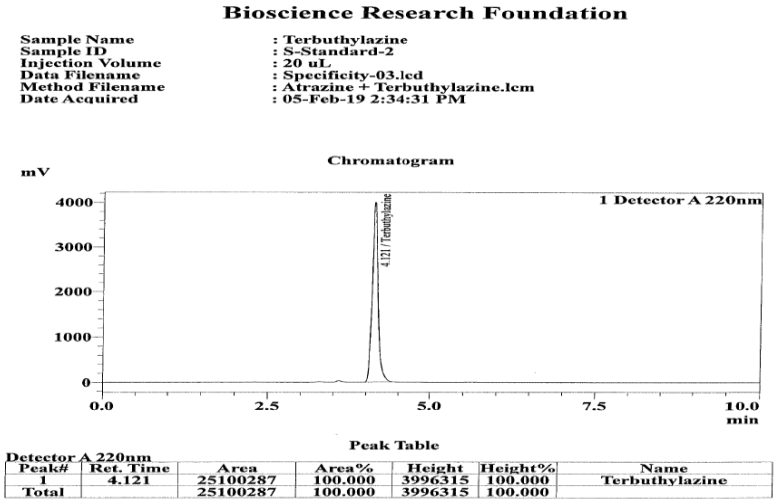

Fig. 2: Typical Chromatogram for Terbuthylazine

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

The specificity of HPLC method for Atrazine and Terbuthylazine were 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 the test substance

3.2 Linearity

3.2.1 Preparation of Standard Stock Solution and working standard: The standard solution, (100 mg/L) was prepared from the standard stock solution (1000 mg/L). The serial dilutions were made to prepare further concentrations such as 10, 20, 30, 40, 50 and 60 mg/L separately. The dilution details are presented in table 1.

Table 1: Dilutions (Atrazine and Terbuthylazine Reference Standard)

Standard Code	Stock concentration (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final concentration (mg/L)
Stock	1000	5	50	100
STD-1	100	1	10	10

STD-2	100	2	10	20
STD-3	100	3	10	30
STD-4	100	4	10	40
STD-5	100	5	10	50
STD-6	100	6	10	60

The prepared standard solutions were injected by an auto sampler into 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 Atrazine Reference Standard

Std. Code	Concentration (mg/L)	Replication	Ref. Std. Area	Mean Std. Area
Std-1	10	R1	2436594	2439631
		R2	2442667	
Std-2	20	R1	4728776	4728319
		R2	4727861	
Std-3	30	R1	7059315	7060082
		R2	7060848	
Std-4	40	R1	9700697	9646881
		R2	9593064	
Std-5	50	R1	11815251	11812033
		R2	11808815	
Std-6	60	R1	14105573	14070620
		R2	14035666	
			Intercept	93638.5000
			Slope	234265.3929
			Correlation Coefficient	0.9998

Table 3: Linearity of Terbuthylazine Reference Standard

Std. Code	Concentration (mg/L)	Replication	Ref. Std. Area	Mean Std. Area
Std-1	10	R1	2023800	2019880
		R2	2015960	
Std-2	20	R1	3902360	3898171
		R2	3893982	
Std-3	30	R1	5801442	5802602
		R2	5803761	
Std-4	40	R1	7974950	7929817
		R2	7884684	
Std-5	50	R1	9550269	9669978
		R2	9789686	
Std-6	60	R1	11585088	11543767
		R2	11502446	
			Intercept	104495.3333
			Slope	191605.9143
			Correlation Coefficient	0.9997

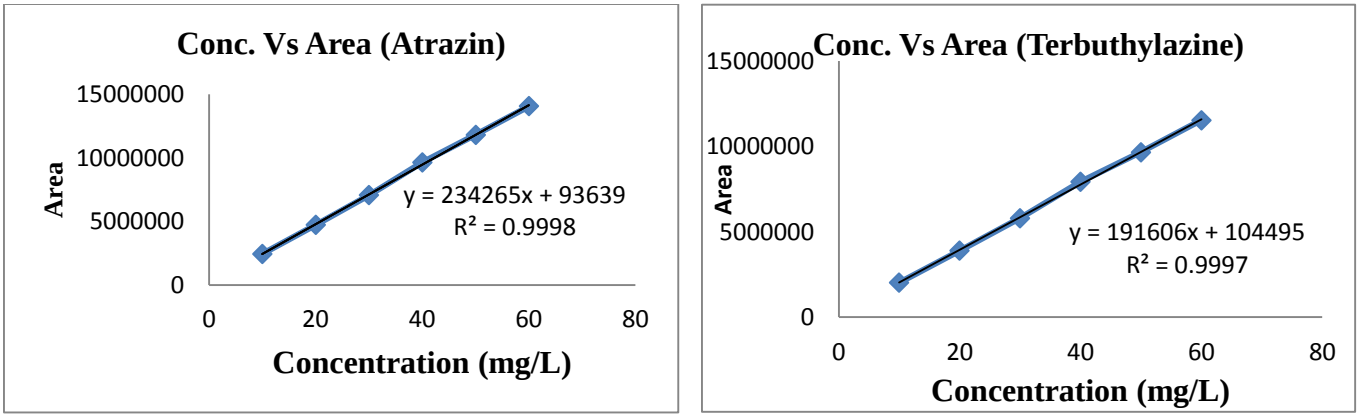

Fig. 1: Linearity Curve for Atrazine and Terbuthylazine

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 aliquot of 3.0 ml specificity sample solution (100 mg/L) was diluted in 10 ml of the mobile phase. This was equivalent to 30 mg/L. The prepared solution was used for precision determination. The prepared concentration was injected 5 times and %RSD was calculated. The prepared solutions were injected into HPLC and %RSD was calculated and the results are presented in table 4.

Table 4: Precision (Atrazine)

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P) %	A.I. Content (%)
Std-R1	10.0	319269	319533.0		95.9	-
P1		600792		142.50		12.65
P2		600636		142.70		12.63
P3		600031		142.80		12.61
P4		600490		142.90		12.61
P5		600447		142.80		12.62
Std-R2		319797				-
					MEAN	12.63
					SD	0.018
					% RSD	0.141

Table 5: Precision (Terbuthylazine)

Sample ID	Std. Conc. (mg/L)	Std. / Sample Area	Average Std. Area	Sample Conc. (mg/L)	Purity (P) %	A.I. Content (%)
Std -R1	30	5821823	5799616	30.0	99.5	-
P1		1691554				29.02
P2		1693951				29.06
P3		1697608				29.12
P4		1694363				29.07
P5		1697864				29.13
Std - R2		5777409				-
					MEAN	29.08
					SD	0.046
					% RSD	0.157

4.3 Formula for Active content calculation

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

The % RSD is within limit according to the modified Horwitz equation (Acceptable Limit <1.3 RSD for 100% active content 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 for linearity (5 mg/L) was used as a standard in percent recovery determination.

5.2 Preparation of Fortification Level 1 (30 mg/L)

An aliquot of 3 ml Linearity standard solution (Atrazine and Terbuthylazine) 100 mg/L was transferred in to 10 ml volumetric flask, diluted with Acetonitrile and made up to the mark with the Acetonitrile. The concentration of the prepared solution was equivalent to 30 mg/L of Terbuthylazine & Atrazine respectively. These prepared solutions were used for % recovery determination.

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

An aliquot of 5 ml Linearity standard solution (Atrazine and Terbuthylazine) 100 mg/L was transferred in to 10 ml volumetric flask, diluted with Acetonitrile and made up to the mark with the Acetonitrile. The concentration of prepared solutions was equivalent to 50 mg/L of Atrazine and Terbuthylazine respectively. These prepared solutions were used for % recovery determination. The above preparations were analyzed under HPLC and checked for recovery (%). The results are presented in following table 6 and table 7.

Table 6: Accuracy (Level-1 & 2 Recovery %) of Atrazine

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	10.0	318737	318851.0	-	29.00	-	-
T1R1		925036		29.0115		100.04	99.96
T1R2		921780		28.9094		99.69	

T1R3		924487		28.9943		99.98	
T1R4		925028		29.0113		100.04	
T1R5		925279		29.0192		100.07	
T2R1		1506822		47.2579		98.45	
T2R2		1504947		47.1991		98.33	
T2R3		1507640		47.2835	48.0	98.51	98.50
T2R4		1510372		47.3692		98.69	
T2R5		1508068		47.2970		98.54	
Std-R2		318965		-		-	

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		412998		-		-	-
T1R1		1205981		29.1039		100.36	
T1R2		1204307		29.0635	29.00	100.22	100.32
T1R3		1205056		29.0816		100.28	
T1R4		1204473		29.0675		100.23	
T1R5	10.0	1208039	414370.5	29.1536		100.53	
T2R1		1974404		47.6483		99.27	
T2R2		1967610		47.4843		98.93	
T2R3		1972109		47.5929	48.0	99.15	99.13
T2R4		1973271		47.6209		99.21	
T2R5		1971456		47.5771		99.12	
Std-R2		415743		-		-	

5.4 Example Calculation: **Recovery (Atrazin) - T2R5**

$$Recovery\ Conc.\left(\frac{mg}{L}\right) = \frac{\text{Std. Conc.}\left(\frac{\text{mg}}{\text{L}}\right) \times \text{Sample area}}{\text{Mean Std. Area}} = \frac{30 \times 14176000}{7068165} = 60.17$$

$$\text{Recovery (\%)} = \frac{\text{Recovery Conc. (mg/L)}}{\text{Fortified Conc (mg/L)}} = \frac{60.17}{60.0} \times 100 = 100.28\%$$

6. LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)

From the Linearity Standard Solution concentration of 10 mg/L was used in these LOD & LOQ determinations. From this solution 1 mg/L solution was prepared and further diluted to get the 0.01 & 0.1 mg/L concentration solutions were prepared. The dilution details were given in the Table No. 8, and the results are presented in following Table 9 & 10.

Table 8: Dilutions (LOD & LOQ)

Stock Concentration (mg/L)	Dilution Volume (ml)	Final Volume (ml)	Final Concentration (mg/L)
1.0	1	10	0.1
0.1	1	10	0.01

Formula:

$$\text{LOD} = \text{Average} + (3 \times \text{Standard Deviation})$$
$$\text{LOQ} = \text{Average} + (10 \times \text{Standard Deviation})$$

Table 9: Limit of Detection (LOD) and Limit of Quantification (LOQ) Of Atrazine

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1		7046894		-
R1		951		0.004
R2	30	634	6990767	0.003
R3		895		0.004
STD-2		6934639		-
			MEAN	0.0035
			SD	0.00073
			LOD	0.01

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1		7046894		-
R1		27180		0.117
R2	30	24161	6990767	0.104
R3		23974		0.103
STD-2		6934639		-
			MEAN	0.108
			SD	0.00772
			LOQ	0.18

Table 10: Limit of Detection (LOD) And Limit of Quantification (LOQ) Of Terbuthylazine

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	30	5700139	5735571	-
R1		1362		0.0071
R2		1292		0.0068
R3		1354		0.0071
STD-2		5771003		-
			MEAN	0.0070
			SD	0.00020
			LOD	0.01

Sample ID	Std. Conc. (mg/L)	Std./ Sample Area	Average Std. Area	A. I. Content (mg/L)
STD-1	30	5700139	5735571	-
R1		19976		0.104
R2		19851		0.104
R3		19949		0.104
STD-2		5771003		-
			MEAN	0.104
			SD	0.00034
			LOQ	0.11

6.1 Example Calculation: (LOD and LOQ)

6.1.1 Limit of Detection (Terbuthylazine) R1

$$\text{A. I Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc.} \left(\frac{\text{mg}}{\text{L}}\right) \times \text{ Sample Area}}{\text{Average Std. Area}} = \frac{30 \times 1362}{5735571} = 0.0071$$

$$\text{LOD} = \text{ Mean Value } + (3 \times \text{SD})$$

$$= 0.0070 + (3 \times 0.0002) = 0.01$$

6.1.2 Limit of Quantification (Terbuthylazine) R1

$$\text{A. I Content } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Std. Conc.} \left(\frac{\text{mg}}{\text{L}}\right) \times \text{ Sample Area}}{\text{Average Std. Area}} = \frac{30 \times 19976}{5735571} = 0.104 \text{ mg/L}$$

$$\text{LOQ} = \text{Mean Value } + (10 \times \text{SD})$$
$$= 0.104 + (10 \times 0.00034) = 0.11$$

7. ACTIVE CONTENT ANALYSIS OF ATRAZINE AND TERBUTHYLAZINE

7.1 Preparation of Standard solution

An amount of 15 mg of the standard was dissolved in 100 ml of mobile phase and diluted to get 30 mg/L was used as a standard in concentration analysis.

7.2 Preparation of Sample Solutions

The formulation sample (10 mg/L) was prepared and dissolved by sonication and diluted appropriately and injected into HPLC.

$$\frac{\text{Terbuthylazine}}{\text{Atrazine}} \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Concentration of standard } \left(\frac{\text{mg}}{\text{L}}\right) \times \textit{Area of sample} \times \textit{Dilution solution}}{\text{Area of standard solution}}$$

8. CONCLUSION

- **Specificity:** The blank, standard and the sample peaks were not co-eluted each other. The Chloro triazine-based compounds Atrazine and Terbuthylazine were separated well with this simple HPLC (Reverse Phase) method. Hence the specificity was achieved as per the guideline SANCO 3030/99 Rev.4 requirement.
- **Linearity:** The Linearity correlation coefficient is achieved NLT 0.99 as per (SANCO 3030/99 Rev.4
- **System Precision:** The system precision is achieved as the % RDS for 5 replicates observed as 0.1% for Atrazine and Terbuthylazine, hence the minimum requirement of the (SANCO 3030/99 Rev.4 was NMT 15% RSD was achieved
- **System Recovery:** The system recovery 92% to 101 % were achieved for, hence the minimum requirement of the (SANCO 3030/99 Rev.4).
- **Details of the Laboratory work were carried out.**
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