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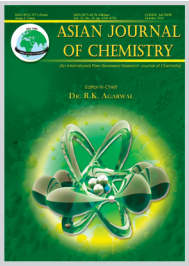

Development and Validation of RP-HPLC and UV Methods of Analysis for Metribuzin in Its Formulation

C. SWARNA, K. BABU NAIDU and N.V.S. NAIDU*

Department of Chemistry, S.V. University, Tirupati-517 502, India

*Corresponding author: E-mail: giddaluriswarna@gmail.com

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A RP-HPLC and an UV Spectrophotometric assay method were developed and validated for quantitative determination of metribuzin in formulation Tata metri. The chromatography was carried out on a waters symmetry C8 (150 mm × 4.6 m, 5 µm) column with potassium dihydrogen orthophosphate and acetonitrile (60:40 v/v) as mobile phase at 297 nm detector wavelength. The UV method was performed at 297 nm using methanol as solvent. The linearity was established in the range of 2 to 12 µg/mL and 5 to 50 µg/mL for HPLC and UV method respectively. The HPLC method was accurate and precise for the formulation 99.38 to 100.79 %. The UV method also correlated well with HPLC for the analysis of metribuzin in its formulation.

Key Words: Metribuzin, Reversed phase high performance liquid chromatography, Ultraviolet spectrophotometry, Analytical method validation, Formulated form of metribuzin (Tata metri).

INTRODUCTION

Pesticides have been used extensively as a strategy to improve agricultural productivity, but their use causes environmental and toxicological risks and groundwater contamination by herbicides has been a major concern in recent years. Metribuzin is available in the form of liquid suspensions, water dispersible granules and dry flowable formulations[1,2]. Metribuzin was registered as a pesticide for the first time in the N.S in 1973[3]. Metribuzin (4-amino-6-*tert*-butyl-3-methylthio-1,2,4-triazin-6(4*H*)-one) belongs to the class of triazines that are widely used for weed control[4]. It is a selective triazinone that inhibits photosynthesis and is used for the pre- and post-emergence control of many grasses and broad-leaved weeds in soybeans, potatoes, tomatoes, sugarcane, alfalfa, asparagus, maize and cereals at 0.07-1.05 kg active in gradient (a.i)/ha[5]. Metribuzin is applied by various methods including aerial and ground applications and chemigation[6]. Metribuzin is weakly sorbed to soil therefore, leaches easily to lower soil profiles. Its persistence in the soil varies between 80 and 90 days[7]. In general, metribuzin is relatively mobile in sandy and mineral soil but immobile in soil with high organic matter[8]. It is slightly toxic *via* the oral route, with reported oral LD_{50} values of 1090-2300 mg kg^{-1} in rats[9].

Analysis of metribuzin has mainly been accomplished by different chromatographic methods such as liquid chromatography[10], gas chromatography[11,12], micellar electrokinetic chromatography[13], Solid phase extraction and sample stacking-micellar electrokinetic capillary chromatography[14], capillary gas chromatography[15], capillary zone electrophoresis[16], molecularly imprinted polymer[17]. Polarography and voltammetry have been used to investigate the mechanisms of electrochemical reduction[18] and photochemical degradation[19] of the related herbicide metamitron. Only one work[20] has described the electrochemical reduction of metribuzin in 30 % v/v acetonitrile-water solution. Although DPP has been used for the determination of metribuzin in soil[21], high performance liquid chromatography[22,23] and thin layer chromatography[24] methods were more frequently employed for the analysis of metribuzin and its metabolites in different metrices.

The process of reduction and electroanalytical determination of metribuzin has been studied by polarographic techniques[24,25]. Only one spectrophotometric method for the determination of metribuzin was reported[26]. This paper describes a validated HPLC and UV spectrophotometry method for the quantitative determination of metribuzin in its formulation.

EXPERIMENTAL

Standard metribuzin was kindly supplied by Tata, Mumbai, India. Acetonitrile (HPLC grade), potassium dihydrogen orthophosphate (HPLC grade) were purchased from SD Fine Chem., Mumbai, India. Triple distilled water used for HPLC and UV method respectively. Formulated product of metribuzin was purchased from local market (Tata Metri).

Analytical conditions: The HPLC method was performed on a Shimadzu system equipped with LC-20 ATV pump, SPD-20 AVP UV detector and Rheodyne injector system fitted with 20 µL loop. The HPLC analysis was performed on reversed phase high-performance liquid chromatographic system with isocratic elution mode using a mobile phase of acetonitrile buffer (40:60 v/v) on waters symmetry C8 column (150 mm × 4.6 mm, 5 µm particle size) with 1 mL/min flow rate at 297 nm using UV detector. Spinchrom 21 CFR software was used for the data interpretation. The UV spectrophotometric method was performed on a UV-visible spectrophotometry (Model 117 systronics) using 1 cm quartz cells (systronics), systronics software was used for absorbance measurements. The UV spectrophotometric method was performed at 297 nm using methanol as solvent for the preparation of standard and sample solutions.

Preparation of standard solutions

HPLC method: 10 mg of accurately weighed standard metribuzin was dissolved and made upto mark with mobile phase in a 100 mL of volumetric flask to get primary stock solution of 100 µg/mL. Serial dilutions were made to obtain, 2, 4, 6, 8, 10, 12 µg/mL using mobile phase. All solutions were filtered through 0.45 µ membrane filter prior to use.

UV method: About 100 mg of accurately weighed standard metribuzin pure dissolved in 50 mL of methanol and made upto mark with methanol solution, in 100 mL volumetric flask, to give primary (stock solution a) of 100 mg/mL from the above stock solution 10 mL of aliquot was pipette out in 100 mL volumetric flask and the volume was made up to mark with methanol to obtain the final concentration of 100 µg/mL (stock solution b).

Preparation of the sample solutions

HPLC method: The powder equivalent to 10 mg of formulated metribuzin (Tata Metri), was accurately weighed and transferred into a 100 volumetric flask made up to mark with mobile phase. This solution was filtered through 0.45 µ membrane filter and diluted suitably using mobile phase to obtain 100 mg/mL solution.

UV method: The powder equivalent to 100 mg of metribuzin was accurately weighed and transferred into a 100 mL volumetric flask. To this 50 mL of methanol solution was added and kept for 10 min with occasional shaking to disperse and dissolve the contents. The volume was made upto 100 mL with methanol solution to give 1000 µg/mL of metribuzin solution. This solution was filtered through 0.45 µ membrane filters and further diluted with methanol solution to give 100 mg/mL.

Method validation: The methods were validated according to international conference on harmonization (ICH) guidelines for validation of analytical procedures.

Linearity: Six concentrations of the standard solutions in 2-12 µg/mL range were analyzed by HPLC. Calibration curves were constructed by plotting average peak areas *versus* concentrations (Fig. 1). Eight concentrations of the standard solutions in the range of 5-50 µg/mL were analyzed for UV method. Calibration curves were constructed by plotting average absorbance versus concentrations (Fig. 2). Linearity

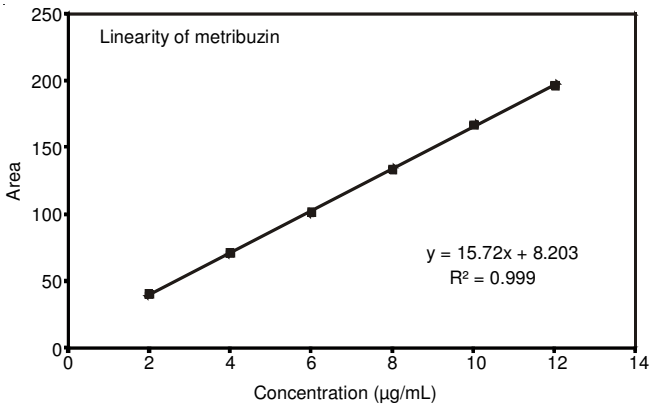

Fig. 1. Calibration curve for metribuzin (HPLC Method)

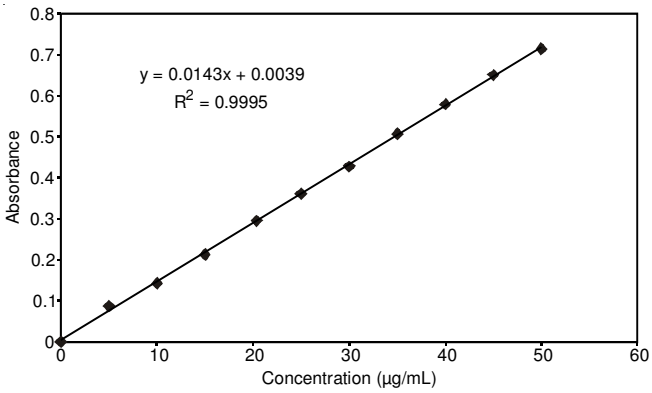

Fig. 2. Calibration curve for Metribuzin (UV Method)

was determined by regression equations for both methods. This experiment was repeated six times for both methods.

Precision: Repeatability was evaluated by analyzing five independent metribuzin standard solutions (10 µg/mL for HPLC method and 50 µg/mL for UV method). The intermediate precision was evaluated on three independent metribuzin standard solutions per day for three different days (Table-1).

Accuracy (by standard addition method): For the HPLC method, an accurately weighed amount of powder (formulation) equivalent to 10 mg of metribuzin was transferred to 50 mL volumetric flask dilute to volume with mobile phase. Aliquots of 0.9, 1.1, 1.3 mL of metribuzin standard solution (100 µg/mL) and transferred to 100 mL volumetric flask and dilute to 100 mL with mobile phase and to make up to give a final concentration 9, 11, 13 µg/mL. For the UV method, an accurately weighed amount of formulated powder equivalent to 100 mg of metribuzin was transferred to 100 mL volumetric flask and dissolved in methanol. Aliquots of 2,4, 6 mL of this solutions were transferred into 100 mL volumetric flask and made upto mark with methanol and give final concentration 20, 40, 60 µg/mL. All solutions were prepared in triplicate and assayed. The percent recovery of added metribuzin standard was calculated (Table-2).

Limit of detection (LOD) and limit of quantification (LOQ): The parameters LOD and LOQ were determined using signal to noise (S/N) ratio.

Stability of standard and sample solution: The standard solution of metribuzin (100 µg/mL for HPLC method and 100 µg/mL for UV method) and sample solution of metribuzin formulations (100 µg/mL for HPLC method and 100 µg/mL

TABLE-1
REGRESSION ANALYSIS AND SYSTEM SUITABILITY PARAMETERS FOR THE QUANTIFICATION OF METRIBUZIN BY HPLC AND UV

Parameter	HPLC Method	Parameter	UV Method
Retention time (t) min	4.177	λ_{max} (nm)	297
Linearity range (µg/mL)	2-12	Beer's law limits (µg/mL)	5-50
Theoretical Plates (n)	9711		
Plates Per meter (N)	64740	Molar absorptivity (L mol^{-1} cm^{-1})	$0.248x10^4$
Height equivalent to theoretical plate (HETP)	0.015	Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.086
Peak asymmetry	0.0019	–	
Regression equation (y = a + bc)		Regression equation (y = a + bc)	
Slope (b)	15.727	Slope (b)	0.0143
Intercept (a)	8.203	Intercept (a)	0.0039
Standard deviation (SD)	0.0088	Standard deviation (SD)	0.0017
Correlation coefficient (r^2)	0.9998	Correlation coefficient (r^2)	0.9995
Relative Standard deviation* (%RSD)	0.21	% Relative Standard deviation* (% RSD)	1.23
Intermediate Precision** (% RSD)	0.23	Intermediate Precision** (% RSD)	1.22
LOD (µg/mL)	0.137	LOD (µg/mL)	0.356
LOQ (µg/mL)	0.428	LOQ (µg/mL)	1.188
Percentage of Errors (Confidence limits)			
0.05 level	± 0.983	0.05 level	± 0.00178
0.01 level	± 1.542	0.01 level	± 0.00278

*RSD of 6 independent determinations in a day; **RSD of 9 independent determinants (3 independent samples per day for 3 days).

TABLE-2
ACCURACY TEST RESULTS FOR METRIBUZIN FORMULATION BY HPLC AND UV

Method	Product	Conc. of pesticide added (µg/mL)	Amount found (µg/mL)	% of Recovery*	SD (%)	RSD (%)
HPLC	Tata metri	9	9.09	100.79	0.045	0.501
	Tata metri	11	10.95	99.55	0.017	0.157
	Tata metri	13	12.92	99.38	0.117	0.194
UV	Tata metri	20	20.10	100.50	0.026	0.131
	Tata metri	40	39.98	99.95	0.017	0.042
	Tata metri	60	59.99	99.98	10.022	0.037

*Average of 3 determinations.

TABLE-3
STABILITY OF THE STANDARD SAMPLE SOLUTIONS OF METRIBUZIN

Time interval (h)	RP-HPLC Method				UV Method			
	Standard solution		Sample solution		Standard solution		Sample solution	
	Recovery (%)*	Difference (%)	Recovery (%)*	Difference (%)	Recovery (%)*	Difference (%)	Recovery (%)*	Difference (%)
0	100.00	–	100.00	–	100.00	–	100.00	–
24	100.11	-0.11	100.21	-0.21	99.52	0.48	99.00	1.00
48	99.94	0.06	99.82	0.18	98.55	1.45	98.25	1.75

*Average of 3 determinations.

for UV method) were prepared in triplicate and analyzed after 48 h by storing the solutions at room temperature (Table-3).

Analysis of metribuzin formulation by RP-HPLC and UV methods: Metribuzin formulated form (Tata Metri) was analyzed by optimized RP-HPLC method. The product was analyzed by six independent determinations. The same product was analyzed by optimized UV method with six independent determinations.

RESULTS AND DISCUSSION

Optimization of HPLC method: Optimization of mobile phase was performed based on peak symmetric, peak width and run time. The mobile phase of buffer and acetonitrile (60:40 v/v) was found to be satisfactory. Fig. 3 shows typical chromatogram obtained from the standard solution of metribuzin using the proposed method. The retention time

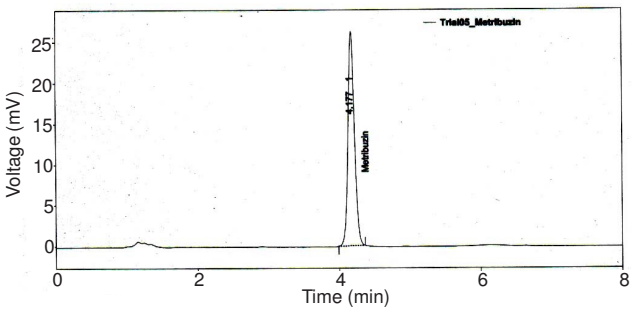

Fig. 3. Chromatogram of standard metribuzin

observed (4.177 min) permit a rapid determination of the pesticide, which is important for routine analysis. System suitability parameters for this method are reported in Table-1. The parameters were within the acceptance limits.

TABLE-4
ASSAY RESULTS OF MARKETED METRIBUZIN FORMULATION BY HPLC AND UV

Formulation name labeled amount (mg)	HPLC Method				UV Method			
	Sample conc. found (µg/mL)*	Recovery (%)	SD (%)	RSD (%)	Sample conc. found (µg/mL)*	Recovery (%)	SD (%)	RSD (%)
Tata metri 70 % wet. table powder	70.12	100.17	0.37	0.53	69.32	99.02	0.31	0.44
	70.14	100.20	0.38	0.54	69.80	99.71	0.30	0.44
	71.10	101.57	0.42	0.59	68.48	98.28	0.39	0.57
	69.89	99.84	0.50	0.72	70.10	100.14	0.23	0.32
	69.74	99.62	0.28	0.41	69.86	99.80	0.42	0.61
	69.94	99.91	0.37	0.54	69.54	99.34	0.33	0.48

*Average of 3 determinations.

Validation of HPLC method: The described reversed phase HPLC method was found to be specific for metribuzin as none of the excipients interfered with the estimation of metribuzin. The method was found linear over the range of 0.2-12 (µg/mL) (Fig. 1). The LOD and LOQ were found to be 0.137 µg/mL and 0.4280 µg/mL, respectively indicating high sensitivity of the method. The results for accuracy and precision are summarized in Tables 1 and 2. The results of recovery studies indicate a high agreement between the true value and the estimated value.

Validation of UV method: The proposed UV spectrophotometric method was found to be specific for analysis of metribuzin in its formulation as no interference was observed at 297 nm. Hence, the UV method permits a rapid and economical quantification of metribuzin in formulation.

The calibration curves were constructed in the range of 5 to 50 µg/mL (Fig. 2). Beer's law was obeyed over this concentration range. The LOD and LOQ were found to be 0.356, 1.188. The repeatability was 1.23 and 1.22, respectively, demonstrating high precision of the method. The accuracy of the proposed method by standard addition method was determined formulations and the mean recovery was found to be 100.50 % (Table-2). The standard and sample solutions were stable for 48 h (Table-3).

Assay of marketed metribuzin formulations: Results of assay on formulations of metribuzin by proposed HPLC and UV method is reported in Table-4. The assay results of proposed RP-HPLC and UV methods were compared using student's t-test does not reveal significant difference between the experimental values obtained in the standard and sample analysis by the two methods.

Conclusion

The HPLC and UV methods for the determination of metribuzin in its formulation was found to be simple, rapid, precise, accurate and sensitive. A good agreement was observed between HPLC and UV method. The validated HPLC and UV methods can be used for the pesticide analysis in routine quality control for bulk and formulations.

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