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Development and validation of spectrophotometric method for the determination of picloram

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ABSTRACT

A simple and sensitive visible spectrophotometric method for the assay of Picloram has been developed. In this method describe the interaction of Picloram, with the oxidative coupling reaction of Picloram with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of ferric chloride in an acidic medium, which results in a green colored product with absorption maxima at 625 nm. Regression analysis of Beer's law plot showed good correlation in the concentration range of 10-60 µgml^{-1}. Different variables affecting the reaction were studied and optimized. The proposed method was applied successfully for the analysis of Picloram in its technical grade, chemical mixtures and environment samples. No interference was observed from common chemical mixtures and environment samples.

Keywords: Picloram, Spectrophotometry, MBTH, Ferric chloride.

INTRODUCTION

Picloram (4-amino-3, 5, 6-trichloropicolinic acid) is a selective systemic herbicide, in the chemical class of pyridine compounds, used worldwide to control most annual and perennial broad-leaved weeds in lawns, turf, pastures, rights-of-ways and various crops, such as wheat, barley and oats[1].

Picloram kills or damages annual and perennial broadleaf herbs and woody plants. It acts as an “auxin mimic” or synthetic growth hormone that causes uncontrolled and disorganized growth in susceptible plants. Picloram does not bind strongly with soil particles and is not degraded rapidly in the environment, allowing it to be highly mobile and persistent (half-life of picloram in soils can range from one month to several years). In soils, picloram is degraded primarily by microbial metabolism, but it can be degraded by sunlight when directly exposed in water or on the surface of plants or soil. Picloram can move off-site through surface or subsurface runoff and has been found in the groundwater of 11 states. Picloram may also “leak” out of the roots of treated plants, and be taken up by nearby, desirable species[2-5]. These pesticides are used in the form of salts or esters as active components in different pesticide formulations individually and in mixtures. These formulations, besides the active components, generally also contain one or more inert ingredients, such as liquid hydrocarbons, ethylene glycol, diethylene glycol monoethyl ether, poly glycol, ethanol etc.[6]AOAC for determination of picloram provides the only official method for the determination of picloram and 2, 4-D (2,4-dichlorophenoxy acetic acid) in mixtures in pesticide formulations, by applying a liquid chromatographic method.[7] For analytical purposes, Volta metric method has been proposed for the determination of picloram. The voltametric behaviour and determination of picloram, a member of a pyridine herbicide family, was for the first time investigated on a boron doped diamond film electrode using cyclic and differential pulse voltammetry[8]. The influence of supporting electrolyte and scan rate on the current response of picloram was examined to select the optimum experimental conditions. With regard to UV-Vis spectrophotometric methods; derivative modality has been used for its determination in mixtures. Derivative spectrophotometric method was developed for the determination of the herbicides picloram and triclopyr in mixtures[9]. Differential elution from the bonded phase sorbent is used to cleanly separate mixture of picloram and 2,

4 D[10]. The advantages of solid phase extraction (SPE) over liquid-liquid phase extraction include decreased use of and exposure to hazardous materials, shorter time requirements and no hindrance of the extraction by the formation of emulsions (Johnson et al, 1991). Herbicide extraction by SPE has also been reported for picloram in water and soil[11]. Solidphase extraction using odadecyl (C&bonded porous silica columns has been used for herbicide extraction and cleanup (Junk and Richard, 1988; Huang and Author to whom correspondence should be addressed.

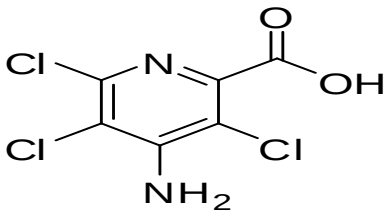

Figure: 1.0 Chemical structure of Picloram

Mol. Formula: $C_6H_3Cl_3N_2O_2$ Mol. Weight: 241.46 grams

Herbicide extraction by SPE has also been reported for picloram in water and.soil (Wells, 1986; Wells and Michael, 1987; Michael et al., 1989) and for dicamba in water (Arjmand et al., 1988). The degradation of the picloram, a widely used herbicide, has been undertaken by the electrochemical advanced oxidation process, namely electro-Fenton in aqueous solution. This process generates catalytically hydroxyl radicals that are strong oxidizing reagents for the oxidation of organic substances[17]. Herbicides containing Picloram are sold under a variety of brand names, Dow Chemicals and now Dow AgroSciences sell herbicides containing it under the brand name Tordon and Grazon. It may be used in formulations with other herbicides such as bromoxynil , atropine , diuron , 2, 4-D, MCOA, tricloroyr and atrazine among others . It is also compatible with fertilizers[12]. However, there is no one reported UV- Visible spectrophotometric method for the analysis of Picloram using oxidative coupling of picloram with MBTH reagent in its technical grade and chemical mixtures and environment samples (water and food grains). This describes a validated UV- visible spectrophotometric method for the quantitative determination of Picloram. Functional group used for color development of Picloram was primary amine group.

The results obtained in this method were based on complex formation reaction of Picloram with Oxidative coupling reaction with MBTH / Ferric chloride The author has developed UV- Visible spectrophotometric method based on the use of method, without use of any interference. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in the various guidelines.

MATERIALS AND METHODS

Solvent

Methanol was used as a Solvent.

Preparation of standard stock solution

Accurately weighed 100 mg of Picloram was dissolved in 40 ml of Methanol in 100 ml volumetric flask and volume was made up to the mark. i.e. 1000 (µg ml^{-1}) (Stock solution A). From the above stock solution A 10 ml of solution was pipetted out into 100 ml volumetric flask and the volume was made up to the mark with methanol to obtain the final concentration of 100 µg ml^{-1}(Stock solution B).

Preparation of calibration curve

Fresh aliquots of Picloram ranging from 1 to 6 ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 10 to 60 µg ml^{-1}.To each flask 1ml of (0.2%) MBTH solution was added followed by 1ml of (0.7%) Ferric chloride solution and resulting solution was heated for 15 min and finally 1ml (0.5N) Hydrochloric acid solution was added. The solutions were cooled at room temperature and made up to mark with Methanol. The absorbance of Green colored chromogen was measured at 625 nm against the reagent blank. The color species was stable for 32hours. The amount of Picloram present in the sample solution was computed from its calibration curve.

Procedure for formulations

An accurately weighed portion of the powder equivalent to 100 mg of Picloram was dissolved in a 100 ml of Methanol and mixed for about 5 min and then filtered. The Methanol was evaporated to dryness. The remaining portion of solution was diluted in a 100 ml volumetric flask to the volume with Methanol up to 100 ml to get the stock solution A. 10 ml of aliquots was pipette out into 100ml volumetric flask and the volume was made up to the

mark with Methanol to obtained the final concentration of 100 µg ml^{-1} (Stock solution). Subsequent dilutions of this solution were made with Methanol to get concentration of 10 to 60 µg ml^{-1} and were prepared as above and analyzed at the selected wavelength, 625 nm and the results were statistically validated.

Recovery of Picloram from Spiked vegetables

100 g of each vegetable (Potatoes and tomatoes) were spiked with 200 ml chloroform for 5 min. The samples were fortified with different concentration of Picloram in Methanol and blended for 3 min. Chloroform was filtered into 250ml Standard flask through whatmanNo.1 filter paper and the residue was retained. The residue was washed twice with 10ml of chloroform and blended for 2 min. Chloroform extracts were combined and made up to the mark. Known aliquots of the chloroform extracts were used for color development after evaporating chloroform on steam bath. The residue was dissolved in methanol and the amount was determined spectrophotometrically and the results were presented in table -1.6.

Recovery of Picloram from Fortified water samples

After collection of the water samples (Tap and Distilled water minimum volume one liter) the pH of the water samples were adjusted below 4 with 20% sulphuric acid. Then fortified with different concentrations of Picloram dissolved in methanol. Extract each sample in a 250 ml separating funnel with 100 ml Chloroform. The chloroform extract was transferred into a funnel and re extracted the aqueous phase twice with further 50ml of chloroform. The second chloroform extracts were added to the first and washed the combined extract with 0.1M K_2CO_3 then dried the chloroform by passing it through anhydrous Sodium sulphate in a filter funnel and collected the extracts in a 250 ml flask. The chloroform extracts were reduced to 100 ml amount was determined spectrophotometrically. The results obtained were presented in table-1.7.

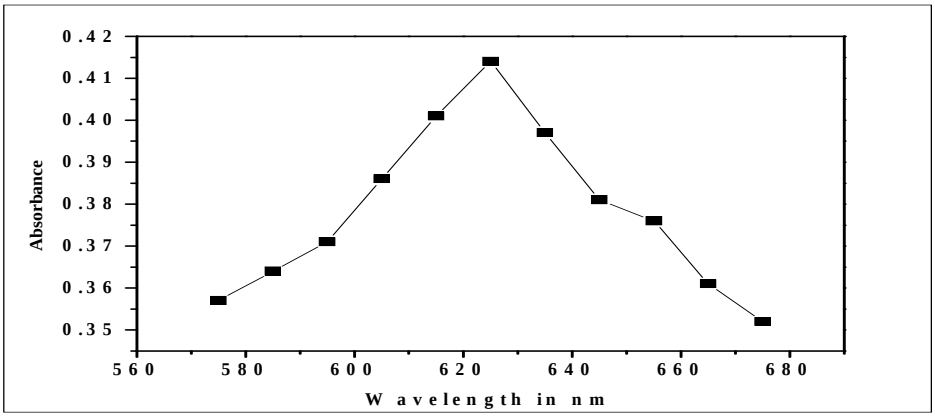

Fig-1.1 Absorption spectrum of Picloram with MBTH /$FeCl_3$

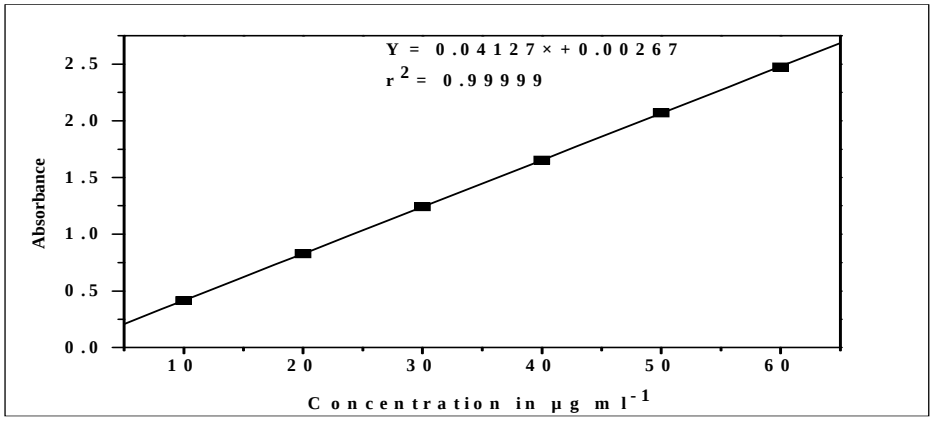

Fig-1.2 Beer's law plot of Picloram with MBTH/$FeCl_3$

Scheme – 1 Reaction Mechanism of Picloram with MBTH

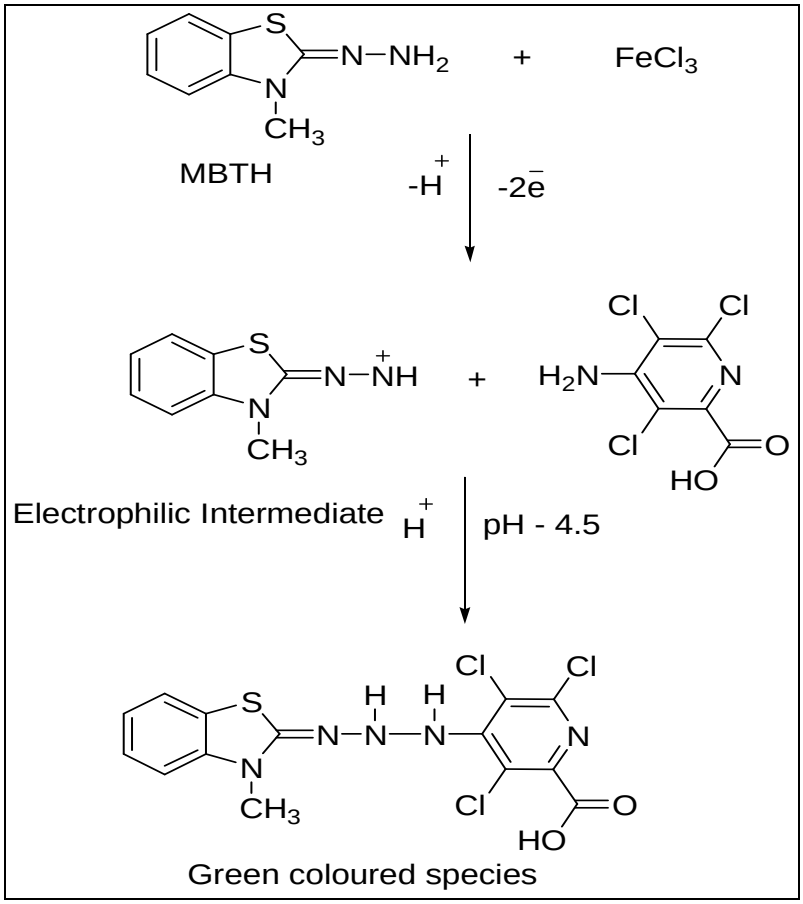

Table-1.0 Optical characteristics and precision by MBTH

Parameter	Visible method
Color	Green
Absorption maxima(nm)	625
Beer's law limits (µg ml^{-1})	10-60
Molar absorptivity (l $mol^{-1}cm^{-1}$)	$0.0414X10^4$
Sandell's Sensitivity (µg cm^{-2})	0.002415
Regression equation (Y*)	
Slope (b)	0.04127
Intercept(a)	0.00267
Standard deviation(SD)	0.00291
Correlation coefficient (r^2)	0.99999
%RSD (Relative Standard deviation)	0.2011
Limits of detection (LOD)(µg ml^{-1})	0.2327
Limits of quantification (LOQ) (µg ml^{-1})	0.7051

%RSD of six independent determinations.

Table-1.1 Assay results of Picloram in formulations by visible method

Name of the Formulation	Formulation in (mg)	Amount found by the proposed method (mg)	Amount found by the reference method (mg)	% Recovery
GRAZON	250	248.02 t= 0.00296 F=1.29315	247.00	99.20
TORDON	250	248.55 t= 0.00295 F=1.29630	248.00	99.42

**t and F- values refer to comparison of the proposed method with reference method.*
**Theoretical values at 95% confidence limits t = 0.00296 F=1.23751*

Table-1.2 Determination of accuracy of Picloram

Amount of Picloram in formulation (mg)	Amount of Standard Picloram added (mg)	Total amount Found (mg)	% Recovery
249.40	200	449.40	99.86
249.35	200	449.35	99.85
249.22	200	449.22	99.83
249.52	250	499.52	99.90
249.32	250	499.32	99.86
249.61	250	499.61	99.92
249.15	300	549.15	99.84
249.69	300	549.69	99.94
249.72	300	549.72	99.95

Table-1.3 Statistical data for accuracy determination

Total amount found (mean)	Standard deviation	% RSD
249.32	0.09292	0.03726
249.48	0.14844	0.05949
249.52	0.32078	0.12855

The results are the mean of five readings at each level of recovery.

Table-1.4 Repeatability data for Picloram at 625 nm

Conc. (µg ml^{-1})	Abs 1	Abs2	Abs3	Mean	Std. deviation	(%) RSD
10	0.414	0.412	0.413	0.413	0.001	0.2421
20	0.828	0.829	0.828	0.82833	0.00058	0.0700
30	1.241	1.242	1.242	1.24167	0.00058	0.0467
40	1.655	1.653	1.654	1.654	0.001	0.0605
50	2.070	2.071	2.070	2.07033	0.00058	0.0280
60	2.475	2.475	2.473	2.47433	0.00115	0.0465

**RSD of six independent determinations*

Table-1.5 Color stability data for MBTH Method

Conc. in µg ml^{-1}	Time in Hours							
20	4	8	12	16	20	24	28	32
	0.828	0.828	0.828	0.828	0.828	0.828	0.810	0.782

Table-1.6 Recoveries of Picloram from spiked Vegetables (Potatoes and Tomatoes)

Sl. No	Amount of picloram added µg ml^{-1}	Average amount found µg ml^{-1}		% Recover		SD		%RSD	
		Potatos	Tomatos	Potatos	Tomatos	Potatos	Tomatos	Potatos	Tomatos
1	0.2	0.191	0.193	95.50	96.50	0.00058	0.001	0.3031	0.5208
2	0.6	0.578	0.582	96.33	97.00	0.001	0.00058	0.1733	0.0996
3	1.0	0.979	0.981	97.90	98.10	0.00153	0.00153	0.1565	0.1557
4	1.4	1.392	1.376	99.42	98.28	0.00058	0.00115	0.0417	0.0835
5	1.8	1.750	1.784	97.22	99.11	0.001	0.001	0.0571	0.0561

Average of five determinations

Table-1.7 Recoveries of Picloram from fortified water samples (Tap and Distilled water)

Sl.No	Fortification level (µg ml^{-1})	Tap water				Distilled water			
		amount found µg ml^{-1}	% Recover	SD	% RSD	amount found µg ml^{-1}	% Recover	SD	% RSD
1	0.2	0.187	93.50	0.001	0.5376	0.190	95.00	0.0005	0.7982
2	0.6	0.583	97.16	0.00058	0.1011	0.581	96.83	0.0005	0.0997
3	1.0	0.964	96.40	0.00058	0.0601	0.959	95.90	0.0005	0.1042
4	1.4	1.392	99.43	0.001	0.0711	1.388	99.14	0.0005	0.0418
5	1.8	1.780	98.88	0.00153	0.0859	1.783	99.05	0.0005	0.0325

Average of five determinations

RESULTS AND DISCUSSION

Optical parameters

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) formed in UV spectrophotometric method of the colored species formed in each specified amounts of Picloram in final solutions 10 µg ml^{-1} was taken and the colors were developed following the above mentioned procedures individually. The absorption spectra were scanned on spectrophotometer in the wavelength region of 380-800 nm against corresponding reagent blanks. The regent blank absorption spectrum of each method was also recorded against distilled water / Methanol.

Parameters fixation

In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were under taken by verifying one parameter at a time and controlling all other parameter to get the maximum color development reproducibility and reasonable period of stability of final colored species formed. The following studies were conducted.

Method

The results obtained in this method were based on oxidation followed by coupling reaction of Picloram with MBTH, Ferric chloride and Hydrochloric acid to form green colored chromogen that exhibited maximum absorption at 625 nm against the corresponding reagent blank. The functional group used for the color development for this method was primary amine group. A schematic reaction mechanism of Picloram with MBTH reagent was shown in (Scheme -1). The effect of various parameters such as concentration and volume of MBTH and strength of acid order of addition of reagents, solvent for final dilution were studied by means of control experiments varying one parameter at a time.

Optical Characteristics

In order to test whether the colored species formed in the methods adhere the beer's law the absorbance at appropriate wavelength of a set of solutions contain different amounts of Picloram and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks or distilled water. The beers law plots of the system illustrated graphically (fig –1.2) least square regression analysis was carried out for the slope, intercept and correlation coefficient, beer's law limits molar absorptivity, Sandals sensitivity for Picloram with each of mentioned reagents were calculated. The optical characteristics are presented in the Table-1.0.

Precision

The precision of each one among the five proposed spectrophotometric methods were ascertained separately from the absorbance values obtain by actual determination of a fixed amount of Picloram in, 10 µg ml^{-1} in final solution. The percent relative standard deviations were calculated for the proposed methods and presented in Table - 1.0.

Analysis of Samples

Commercial formulations of Picloram were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the t and F tests and were found that those proposed methods do not differ significantly from the reported methods and they were presented in Table-1.1.The proposed methods also applied for samples spiked vegetables and water samples for good recoveries are obtained which were recorded in Tables- 1.6& 1.7.

Accuracy

Recovery studies were carried by applying the standard addition method to sample present in formulations for the known amount of Picloram the recovery studies were carried. By applying the same method to samples spiked Vegetables and water samples to which known amount of Picloram correspond to formulations. At each level of recovery five determinations were performed and present in Tables– 1.6& 1.7. The results obtain were compared with expected results and were statistically validated in Table–1.3.

Linearity and Range

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated within a suitable level of precision, accuracy and linearity.

Specificity and Selectivity

Specificity is a procedure to detect quantitatively the analyte in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyte qualitatively in presence of components that may be expected to present in the sample matrix. The excipient in formulations was spiked in a pre-

weighed quantity of pesticides and then absorbance was measured and calculations were done to determine the quantity of the samples.

Repeatability

Standard solutions of Picloram were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measure five times and standard deviation was calculated and presented in Table-1.4.

Interferences Studies

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Picloram under optimum conditions was investigated. None of them interfered in the proposed methods even when they are present in excess fold than anticipated in samples.

Solution Stability

The stability of the solutions under study was established by keeping the solution at room temperature for 32 hours. The results indicate no significant change in assay values indicating stability of Pesticide in the solvent used during analysis. The results are recorded in Table – 1.5.

CONCLUSION

In this study, one UV- Visible spectrophotometric method was developed and validated for the determination of Picloram as bulk, commercial samples and spiked vegetables and water samples. The spectrophotometer instrument is simple and not of high cost, on the other hand interms of simplicity and expense, the proposed methods could be considered superior in comparison with the previously reported methods. The apparatus and reagents used are easily accessible even for the simple laboratories and the procedures do not involve any critical reaction conditions or tedious sample preparation. The methods show no interference from the ingredients usually found in bulk, commercial samples and spiked vegetables and water samples. The statistical parameters and recovery data reveal the good accuracy and precision of the proposed methods. Therefore, it is concluded that the proposed methods are simple, sensitive, reproducible, accurate and precise and can be recommended for routine and quality control analysis of Picloram.

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