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Extractive spectrophotometric method for determination of metribuzin herbicide and application of factorial design in optimization of various factors

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ABSTRACT

A simple extractive spectrophotometric method has been described for the determination of metribuzin herbicide. Metribuzin was reacted with copper and a stable complex in the presence of ammonia (0.2 M) at pH 10.5 was formed. The resulting yellow coloured complex was extracted in chloroform and showed absorption maxima at 340 nm. Beer's law was obeyed in the range of 0.8–25 μg mL^{-1} with molar absorptivity of 5.67×10^3 L mol^{-1} cm^{-1}. The composition of the complex was studied by Job's method of continuous variation and the results indicated that the mole ratio of metribuzin:Cu^{2+} is 2:1. The optimum reaction conditions for complexation and other analytical parameters were evaluated.

A two-level factorial design was also used to determine the effect of different parameters and their interaction on metribuzin:Cu^{2+} complex formed. The method was successfully applied for the determination of metribuzin in commercial formulations and real samples.

1. Introduction

Metribuzin (4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) is a selective triazine herbicide which inhibits photosynthesis of susceptible plant species. It is used for the control of annual grasses and numerous broadleaf weeds in field and vegetable crops mainly potatoes [1]. Metribuzin is weakly sorbed to soil therefore, leaches easily to lower soil profiles. Its persistence in the soil varies between 80 and 90 days [2]. Leaching in soil is increased with increasing soil pH. The mobility of metribuzin in soil is inversely related to the soil adsorptive capacity. In general, metribuzin is relatively mobile in sandy and mineral soil but immobile in soil with high organic matter [3]. It is slightly toxic via the oral route, with reported oral LD50 values of 1090–2300 mg kg^{-1} in rats [4].

Analysis of metribuzin has mainly been accomplished by different chromatographic methods such as liquid chromatography [5–7], micellar electrokinetic chromatography [8], solid-phase extraction and sample stacking-micellar electrokinetic capillary chromatography [9], capillary gas chromatography [10], capillary zone electrophoresis [11] and molecularly imprinted polymer [12]. All these methods are expensive, require long separation times and sometimes need the development of extremely complex gradient for the separation.

No spectrophotometric method is reported so far, for determination of metribuzin. In this work, we report a simple extractive spectrophotometric method for the determination of metribuzin herbicide. The method is based on its complexation with copper in basic media to form a yellow coloured complex with absorption maxima at 340 nm.

2. Experimental

2.1. Apparatus

A Shimadzu UV–Visible Spectrophotometer (UV-1700 Pharmaspec, Japan) with 1-cm matched quartz cells were used for absorbance measurement. WTW pH 422 (W. Germany) model pH meter was used for pH measurement.

2.2. Reagents

All chemicals used were of analytical reagent grade or similar, $CuSO_4 \cdot 5H_2O$, ammonia, and chloroform were purchased from Merck (Darmstadt, Germany), whereas distilled ethanol was used through out the work. Standard reference metribuzin was purchased from Dr. Ehrenstorfer GmbH Germany. Commercial sample containing metribuzin was purchased from the local market.

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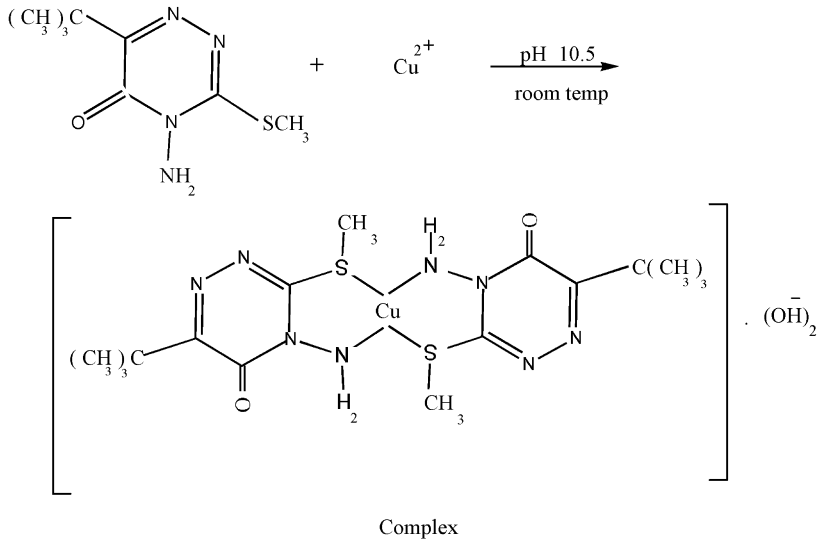

Fig. 1. Proposed reaction mechanism for extractive spectrophotometric determination of metribuzin herbicide.

2.3. Solutions

Ammonia solution (0.2 M). Ammonia solution was prepared by dilution from concentrated ammonia solution.

Copper solution (1000 μg mL^{-1}). 0.395 g of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) was dissolved in 10 mL distilled water and diluted to 100 mL in volumetric flask.

Metribuzin solution. Metribuzin stock solution (1000 μg mL^{-1}) was prepared by dissolving 0.1 g of authentic standard reagent in 50 mL ethanol and diluted with distilled water up to 100 mL in volumetric flask. Working standard of 100 and 10 μg mL^{-1} solutions were prepared by dilution of the stock solution with distilled water.

2.4. Procedure

Metribuzin standard solutions of 0.1–25 μg mL^{-1} were taken in 100 mL volumetric flask. Metal solution of 2 mL from stock solution (1000 μg mL^{-1}) and ammonia solution (0.2 M) was added to each solution and diluted up to the mark with distilled water. These solutions were transferred to separatory funnels and 10 mL of chloroform was added to it and shaken well for 2 min. The two phases were then allowed to separate. The chloroform layer was removed and the absorbance of yellow coloured complex extracted was measured at 340 nm against reagent blank.

2.5. Determination of metribuzin in commercial formulations and real samples of potatoes

Known amount of commercial formulation containing metribuzin was taken and diluted with ethanol up to 50 mL and was filtered to remove any insoluble substances from it. The solutions were then analyzed by the proposed method.

For residue determination in real samples, metribuzin was removed on solubility basis by soaking the potatoes in 50 mL chloroform for two hours and then filtered. The filtrate (chloroform) was evaporated and the residue was redissolved in 50 mL ethanol. The ethanolic solution was then analyzed by the proposed method.

The validity of the method was confirmed by applying standard addition technique. In this method several different concentrations of the standard metribuzin solution (10, 12, 15.5 μg mL^{-1}) were added to the samples to check the recovery. Metribuzin was extracted and determined as mentioned above.

2.6. Experimental design

The effect of different parameters and their interactions on the complex formation was studied according to the 2^3 factorial design [13]. A 2^3 factorial design with a complete repetition performed in one run resulting in a total of eight experiments was chosen for investigating optimum conditions for complexation of metribuzin with metal ion (Cu^{2+}).

3. Results and discussion

Metribuzin has two chemical groups methylthio and amine attached to the triazine ring with the potential to coordinate with transition metals such as Cu^{2+}. Preliminary results confirmed the complexation of metribuzin with copper. The proposed reaction mechanism is given in Fig. 1. Mitribuzin acts as bidentate chelating agent and chelation takes place through both amino and thio groups leading to a five-membered ring with one molecule of metribuzin. Therefore two molecules of metribuzin reacted with Cu^{2+} and formed a stable yellow coloured extractable complex. The resulting complex was extracted in chloroform and was found to have absorption maxima at 340 nm against the reagent blank.

The effect of various experimental parameters on the absorbance of the final yellow coloured complex was studied and is discussed below.

3.1. Effect of pH and ammonia concentration

As the proposed reaction requires basic media, therefore the effect of ammonia concentration was studied in the range of 0.05–1.0 M. Maximum absorbance was observed with ammonia concentration in the range of 0.2–0.4 M (Fig. 2). The effect of pH on the complex formation was also studied in the range of 9.0–11.5 and it was found that maximum complexation is achieved at pH 10.5 (Fig. 3).

3.2. Effect of time

The effect of time on the stability of yellow coloured complex was investigated up to 1.5 h. The complex was found to be stable up to 1 h at room temperature.

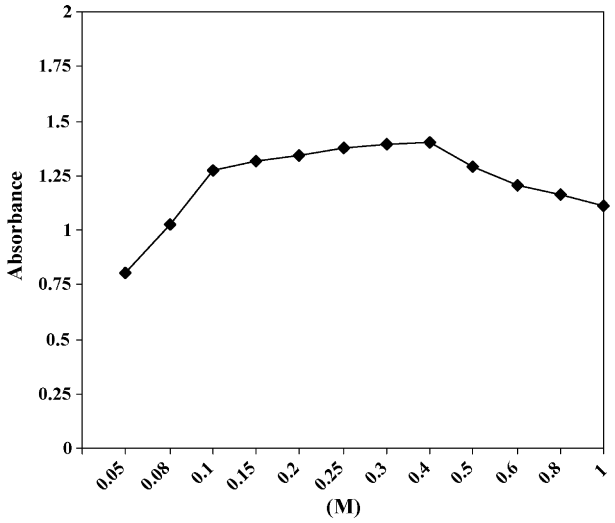

Fig. 2. The effect of ammonia concentration on complex formation.

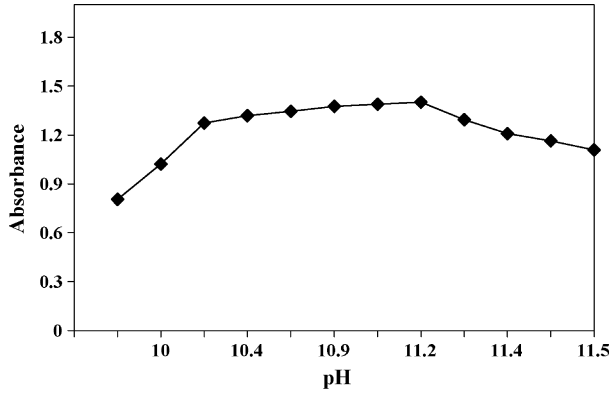

Fig. 3. The effect of pH on complex formation.

3.3. Composition of the complex

The composition of the complex was investigated using Job's method of continuous variations (Fig. 4). Maximum absorbance for the complex was observed at a mole ratio of 3:7 which indicated the formation of 1:2 (Cu^{2+}:metribuzin) complex. The formation constant of the complex was calculated from the data of mole ratio and continuous variations method and was found to be 2.7×10^6.

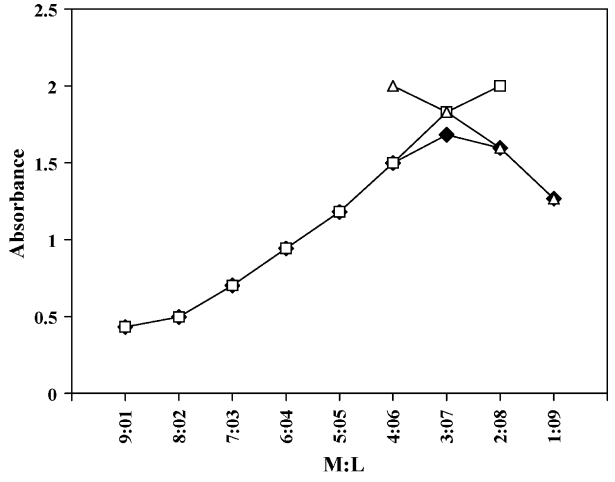

Fig. 4. Formula and stability constant determination by Job's method.

Table 1
Optical characteristics for spectrophotometric determination of metribuzin

Characteristics	Analysis
λ_{max} (nm)	340
Beer's law range ($\mu g\,mL^{-1}$)	0.8–25
Slope	0.047
Limit of detection ($\mu g\,mL^{-1}$)	0.66
Limit of quantification ($\mu g\,mL^{-1}$)	2.2
Molar absorptivity ($L\,mol^{-1}\,cm^{-1}$)	5.67×10^3
Standard deviation	0.2
RSD	5
Correlation coefficient (r^2)	1.0
Stability constant (K_f)	2.7×10^6

Table 2
The 2^3 experimental design

Run	A*	B*	C*	Metal conc ($\mu g\,mL^{-1}$) A*	Ligand conc ($\mu g\,mL^{-1}$) B*	pH C*	*R* absorbance
1	−1	−1	−1	20	20	9.3	0.201
2	+1	−1	−1	40	20	9.3	0.201
3	−1	+1	−1	20	40	9.3	0.201
4	+1	+1	−1	40	40	9.3	0.341
5	−1	−1	+1	20	20	10.5	0.403
6	+1	−1	+1	40	20	10.5	0.403
7	−1	+1	+1	20	40	10.5	1.008
8	+1	+1	+1	40	40	10.5	1.009

3.4. Analytical characteristics

The absorbance concentration curve was found linear over the concentration range of 2–24 $\mu g\,mL^{-1}$. The statistical parameters calculated from the calibration graph are given in Table 1. The linearity of calibration graph is reflected by the high value of correlation coefficient (r^2). The molar absorbtivity of the resulting yellow coloured complex was found to be $5.67 \times 10^3\,L\,mol^{-1}\,cm^{-1}$. The limit of detection (LOD) and quantification (LOQ) were calculated using the minimum concentration at which metribuzin could be detected reliably and it was found to be 0.66 and 2.2 $\mu g\,mL^{-1}$ respectively.

3.5. Experimental design to optimize different variables for maximum complexation reaction

A 2^3 factorial design with a complete repetition performed in one run resulting in a total of eight experiments was chosen for optimization of metribuzin complexation with Cu^{2+}. The optimization design with a threefold repetition for complex formation is given in Table 2.

Three factors were selected for optimization of complexation reaction. The factors and their lower (−) and high level (+) are given in Table 2 and are shown in Fig. 5. The pH, metal ion (Cu^{2+}) concentration and ligand (metribuzin) concentration were varied but the total volume was kept constant for all experiment. The effect of changing a factor from a low level to a high level value was studied on absorbance measurement. For maximum complex formation of metribuzin with metal ion, the highest response was found in the eighth run in Table 2.

Using the data found in Table 2, the effects of the main factors and their interactions with each other were estimated and the results are given in Table 3. The most calculated effect on the absorbance of complex was seen in pH (C = 0.47) and ligand (metribuzin) concentration (B = 0.34). The BC interaction (0.27) has positive effect on complex formation while AC (−0.35) and ABC

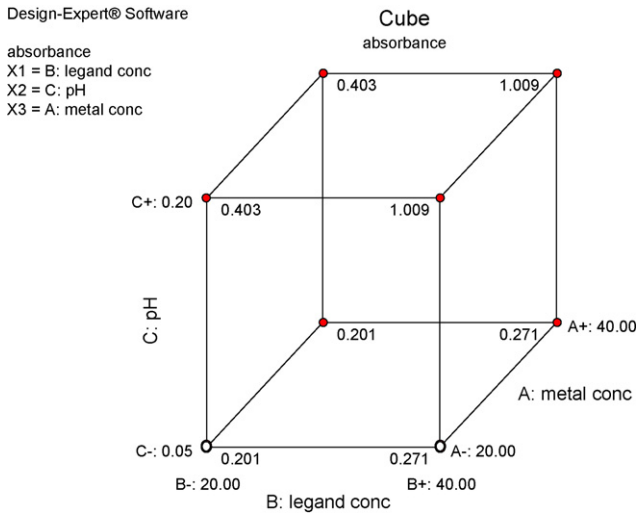

Fig. 5. Cube model graph of the 2^3 factorial design.

(−0.035) have the same negative effect on the complexation reaction.

For statistical confirmation of these effects, the analysis of variance (ANOVA) was carried out and the results are given in Table 4. These results confirmed that B and C are statistically the main factors for complex formation. Note that factor C is significant (0.44) than B (0.23) and the interaction of BC has significant effect (1.0) on complex formation. The optimum conditions for the highest absorbance of metribuzin complex with Cu^{2+} were seen in the eighth run in Table 2.

Fig. 6 represents a contour plot for the response derivations. The line curvature is due to the impact of interactions of the two significant factors which confirm our results.

Table 3
Effect list of main factors and their interactions

Error/included in model	Term	Effect	Sum of squares	% Contribution
Error	A-metal conc	0.035	0.00248513	0.302084
Model	B-ligand conc	0.34	0.22815	27.7332
Model	C-pH	0.47	0.44133	53.6467
Error	AB	0.035	0.00248512	0.302084
Error	AC	−0.035	0.00241512	0.293575
Model	BC	0.27	0.14338	17.4288
Error	ABC	−0.035	0.00241512	0.293575
Lenth's ME 0.197624				
Lenth's SME 0.472952				

3.6. Application

A recovery test was performed on control samples fortified with known concentration of metribuzin solution. Six replicates of the

Table 4
Analysis of variance (ANOVA) for main factors

Source	Sum of squares	df	Mean square	*F*-value	*P*-value, Prob > *F*	
Model	0.81	3	0.27	110.59	0.0003	
B-ligand conc	0.23	1	0.23	93.12	0.0006	
C-pH	0.44	1	0.44	180.13	0.0002	Significant
BC	1	0.14	58.52	0.0016	–	
Residuals	9.800E−003	4	2.450E−003	–	–	
Cor. total	0.82	7	–	–	–	

df = degree of freedom.

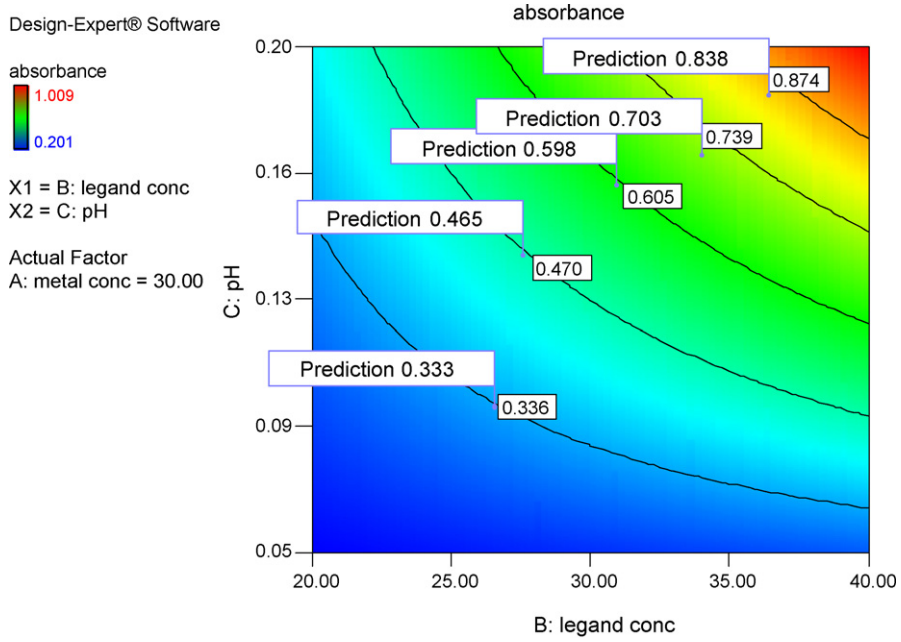

Fig. 6. Contour plot of the predicted and calculated values of response from the design.

Table 5
Percent recovery of Metribuzin from real sample and commercial formulation

Real sample/commercial formulation	Taken ($\mu g\,mL^{-1}$)	Added ($\mu g\,mL^{-1}$)	Found ($\mu g\,mL^{-1}$)	% Recovery
Potatoes	4	10.0	12.6	86.00 ± 0.90
		12.0	15.0	91.66 ± 0.20
		15.5	18.1	90.00 ± 0.40
Commercial formulation	10	10.0	19.8	98.00 ± 0.70
		12.0	21.8	98.00 ± 0.20
		15.5	25.1	97.00 ± 0.40

fortified samples were analyzed using the proposed method. The results are given in Table 5. Recovery values in commercial formulations were found to be 97.0% ±0.4 to 98.0% ±0.2 and in case of real samples the results of the recovery were found to be in the range of 86.0% ±0.9 to 91.7% ±0.2.

The method was also applied for residue determination in potatoes sample. The value for metribuzin residue in potatoes was found to be $0.15 \pm 0.07\ \mu g\,mL^{-1}$.

4. Conclusion

The spectrophotometric method developed for determination of metribuzin in commercial formulations and vegetable samples is simple, sensitive and reproducible as compared to gas chromatographic and liquid chromatographic methods. The optimum conditions for highest absorption due to maximum complexation reaction found experimentally were confirmed using an experimental design. The primary effective factors responsible for the maximum absorbance of complex were found to be metribuzin concentration and pH for complexation reaction.

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