

## DETERMINATION OF DINITROANILINE HERBICIDE IN FOOD SAMPLES AND COMMERCIAL FORMULATIONS USING SPECTROPHOTOMETRIC METHOD

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### ABSTRACT

A simple spectrophotometric method for the determination of herbicides residues of dinitroaniline (trifluralin and pendimethalin) in food samples is described. The proposed method is based on the base hydrolysis of dinitroaniline herbicide with sodium hydroxide. The active ingredient was converted to 2,4 dinitroaniline. Hydrolyzed product was converted to diazonium salt by using sodium nitrite in acidic media. The diazonium salt on coupling with aniline gave red colored azo dye. The absorbance of the azo dye was measured at 460 nm. The reaction conditions were optimized for hydrolysis as well as for the diazotization reactions. The method shows a linear range between 0.5 - 40  $\mu\text{g mL}^{-1}$  with molar absorptivity of  $1.53 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ . The limit of detection and limit of quantification were calculated and found to be 0.21  $\mu\text{g mL}^{-1}$  and 0.71  $\mu\text{g mL}^{-1}$  respectively and Percent recovery was in the range of 86.2 - 92.7%. The method is sensitive, simple and suitable in routine laboratories for application to environmental samples and formulation. The method was successfully applied to soil, water and vegetable (cabbage, potato, onion and garlic samples) collected from crop fields of Peshawar and Swat.

**Key words:** Dinitroaniline herbicides, spectrophotometric method, vegetable samples.

### INTRODUCTION

The development and use of herbicides have played an important role in the increase of agricultural productivity on world level. The use of herbicides has increased the cost of production but on the other hand herbicides pose problems, such as herbicide resistance, weed shift, threat to biodiversity and carryover effect (Khan *et al.*, 2012). Herbicides can provide cost-effective weed control with a minimum of labor however, all other aspects of herbicides should be studied to avoid the ill effects of herbicides. Weeds are plants that are competitive, persistent, and interfere negatively with human activity giving the harmful economic implication of poor weed management (Anderson, 1988). It has been estimated that crop losses due to weed

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competition throughout the world as a whole are greater than those resulting from combined effect of insect pests and diseases (Abbas *et al.*, 2009). Weeds may encourage the development of fungal diseases, provide shelter for pests of all kinds and act as host plants for parasitic nematodes. As a matter of fact, with rising costs of labor and power the use of herbicides in developing countries could be the only acceptable method of weed control in future (Abbas *et al.*, 2009).

Dinitroaniline herbicides (trifluralin, benfluralin and pendimethalin) are used to control many germinating annual grasses and broadleaf weeds. The most successful dinitroaniline, released for the first time for use in food crops in October 1964, was trifluralin that is (2,6-dinitro-N, N-dipropyle-4-tri flura methylbenzene) which has been used extensively as an agricultural herbicide. It is a pre-emergence, soil applied and soil incorporated herbicide and is used for the control of annual grasses and certain broad leaf weeds (Zimdahl, 2010).

Pendimethalin, N-(1-ethylpropyl)-2,6-dinitro-3-4 xylidine) is a dinitroaniline compound which has been used extensively as an agricultural herbicide. The preparations available are Stomp 330, or mixed with other herbicides. Stomp 330 E is used for the control of annual grassy and some broad leaf weeds in corn, soybean, potato, wheat, onion, tomato, cabbage and pepper. It can be applied pre-emergence after seeding maize and rice or with shallow soil incorporation before seeding beans, cotton, groundnuts, and soybeans (Lin *et al.*, 2007). In vegetable crops it can be applied pre-emergence or pre-transplanting. It is also used to control sucker in tobacco (Chen *et al.*, 2007).

Water-soluble herbicides like dinitroaniline generally have low absorption capacities and are more mobile in the environment and therefore more available for microbial metabolism and other deprivation processes (Ramesh and Balasubramanian, 1999). In recent years, dinitroaniline compounds are subjected to increased toxicological and environment concerns, e.g. to cause various physiological changes and endocrine effects in the animal studies including liver parameters, kidney damage and mutagenic effects (Byrd *et al.*, 1994).

Human population is constantly exposed to chemicals present in the environment. Despite the benefit, the use of these kinds of chemicals must be controlled because an important fraction of these herbicides are released into the environment presenting a potential hazard risk. Most of the available investigated methods ELIZA (Hegedus *et al.*, 2000), SFE (Spack *et al.*, 1998), HPLC (Curini *et al.*, 2000), G.C. (Engebretson *et al.*, 2001) for determination of dinitroaniline herbicides residues need expensive instrumentation and

specialized technicians for its smooth running. The main objective of the new method was routine application, minimal cost, enhanced sensitivity, reproducibility and reliability with its subsequent application to environmental and agricultural samples.

## **MATERIALS AND METHODS**

### **Instruments**

UNICO UV-2100 united product and instruments Inc, Dayton; U.S.A (UV/Vis spectrophotometer) with matched cells was used for all spectral measurements. A pH meter model pH-422 (Wissenschaftlich-Technische Werkstätten, W. Germany) was used for pH measurements.

### **Reagents**

All chemicals used were of Analytical Reagent grade or of similar purity. Sodium nitrite, hydrochloric acid, sodium hydroxide and aniline (Merck, Darmstadt, Germany) and distilled ethanol were used during this work. Commercial formulation containing pendimethalin and trifluralin was purchased from the local market. Reference standard pendimethalin was purchased from Dr. Ehrenstofer GmbH, Germany.

### **Solutions Preparation**

- i) Pendimethalin ( $1000 \mu\text{g mL}^{-1}$ ): 0.1g of pendimethalin was dissolved in 1000 mL volumetric flask and diluted up to mark with acetic acid.  $100 \mu\text{g L}^{-1}$  solution was prepared by dilution formula from the stock solution.
- ii) NaOH (2 %): 2 g of NaOH was dissolved in distilled water and diluted up to 100 mL with distilled water in volumetric flask.
- iii) HCl (1M): Concentrated HCl solution was used.
- iv) Nitrite ( $1000 \mu\text{g mL}^{-1}$ ): 0.15 g of sodium nitrite was dissolved in distilled water and diluted up to 100 mL with distilled water in volumetric flask.
- v) Aniline (2%): 2mL of aniline was taken in volumetric flask (100 mL) and diluted up to mark with 50% ethanol.

### **Procedure**

From  $100 \mu\text{g mL}^{-1}$  stock solution of pendimethalin 5 ml was taken in different titration flasks. To each flask 5 mL NaOH (2M) solution was added and heated on boiling water bath for hydrolysis in the range of 10-50 minutes. After completion of hydrolysis time, the contents of the flasks were cooled to room temperature and transferred to volumetric flasks. 5 mL nitrite solution ( $100 \mu\text{g mL}^{-1}$ ) was added, followed by the addition of 1 mL HCl solution and 5 mL of aniline solution (2 %). All the solutions were diluted up to 50 mL with

distilled water. The absorbance of solution was measured at 460 nm against blanks (synthetic) using UNICO-UV-2100 spectrophotometer.

### Statistical analysis

The Molar absorptivity was calculated by using the formula,

$$\text{Molar absorptivity } (\epsilon) = A/C \times L$$

Where A= absorbance, C= concentration ( $\text{mol L}^{-1}$ ), L= path length (centimeter). The calculated value is of  $1.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated for five replicates of the lowest quantifiable concentration  $0.5 \mu\text{g mL}^{-1}$  solution by using the following formula (Table-1),

$$\text{LOD (for concentration)} = 3 \times S/b$$

$$\text{LOQ (for concentration)} = 10 \times S/b$$

Where S = Standard Deviation.

Relative standard deviation (RSD) was calculated by using the following formula,

$$\text{RSD} = \text{Standard deviation} / \text{Mean} \times 100$$

Percent recovery was calculated by using formula given below,

$$\% \text{ Recovery} = \frac{\mu\text{g (found in fortified samples)} - \mu\text{g (found in sample)}}{\mu\text{g of standard added}} \times 100$$

## RESULTS AND DISCUSSION

Pendimethalin (N - (1 - ethylpropyl) - 2, 6 - dinitro - 3 - 4 xylidine) reacted with sodium nitrite in presence of alkali and diazotized with coupling reagent aniline. Mechanism of reaction is shown in Fig. 1. The resultant red colored azo dye was measured at 460 nm (Fig. 2). The effect of various experimental parameters on the absorbance of the final red colored product was studied. All spectral measurements were carried out against reagent blank. The Beer's law was obeyed by the azo dye formed in the range of  $0.25\text{-}20 \mu\text{g mL}^{-1}$ .

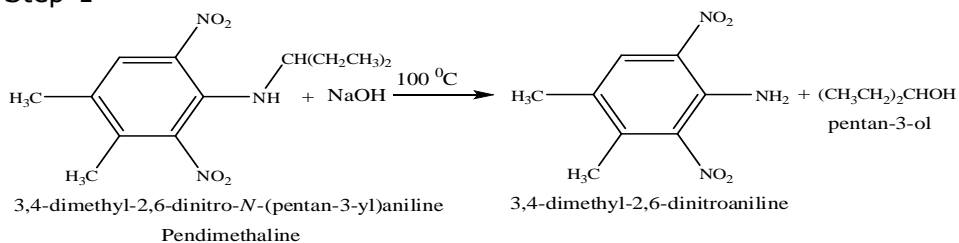
### Optimization of conditions

Effect of heating time on hydrolysis was studied in the range of 5-50 minutes. It was observed that maximum hydrolysis requires 10 minutes heating on boiling water bath; beyond this time it remains constant. Hydrolysis of pendimethalin was performed at different concentrations of sodium hydroxide solution. Maximum hydrolysis was observed by using 8 mL of 2% NaOH solution (Fig. 3).

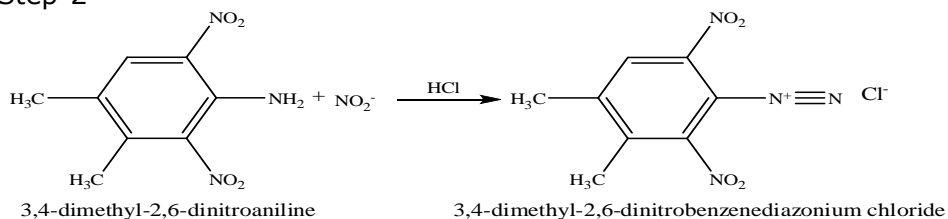
Nitrite concentration was optimized for diazotization in the range of 0.05-0.3% (Fig. 4). Maximum absorbance was observed with 0.2% concentration of nitrite. Similarly the volume of 0.2% nitrite solution was optimized in the range of 2-18mL that 8 mL of 0.2% of

nitrite solution was sufficient for maximum diazotizing and subsequently for maximum formation of azo dye.

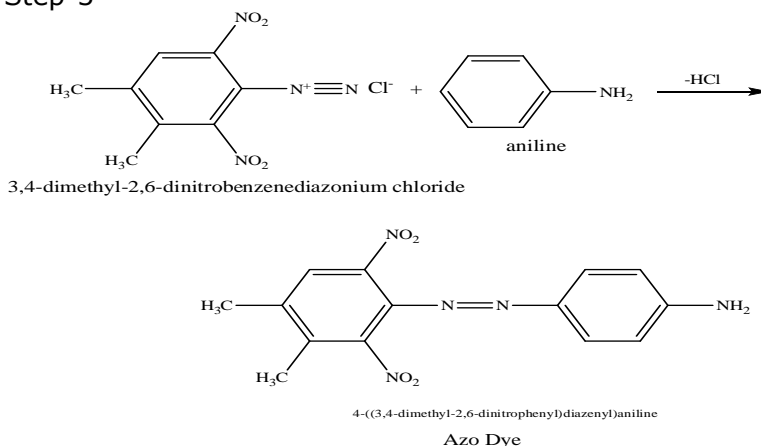
### Step-1



### Step-2



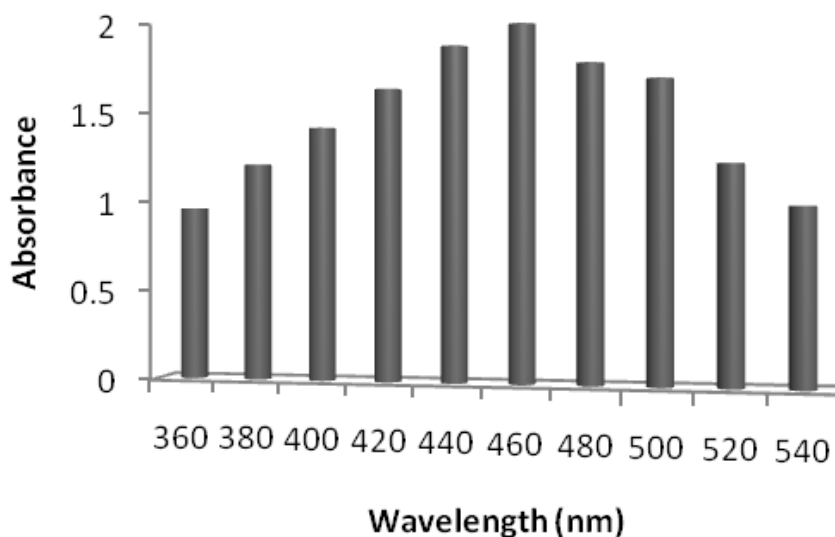
### Step-3



**Figure 1. Proposed reaction mechanisms for spectrophotometric determination of pendimethalin herbicide.**

The concentration of hydrochloric acid required for diazotization of hydrolyzed pendimethalin was optimized in range of 0.1-0.7M. An increase in absorbance was observed with increasing final acid concentration up to 0.4 M of hydrochloric acid solution (Fig. 5). The

diazonium salt was coupled with aniline to form an azo dye. The volume and concentration of aniline solution was optimized in the range of 2-12 mL and it was observed that 8 mL of 2% aniline solution is suitable for maximum absorbance (Fig. 6). The limit of detection (LOD) and quantification (LOQ) was found to be  $0.22 \mu\text{g mL}^{-1}$  and  $0.72 \mu\text{g mL}^{-1}$  respectively with molar absorptivity of  $1.54 \times 10^4 \text{ L. mol mL}^{-1}$ . The validity of the proposed method was checked by standard addition method and the % recovery was found from  $79.0 \pm 0.32$  to  $95.2 \pm 0.29\%$ . The method was applied for residue determination in garlic, water, cabbage and potato samples and found  $1.37 \pm 1.24$ ,  $1.11 \pm 2.18$ ,  $1.41 \pm 1.26$  and  $1.32 \pm 1.18 \mu\text{g g}^{-1}$ , respectively.



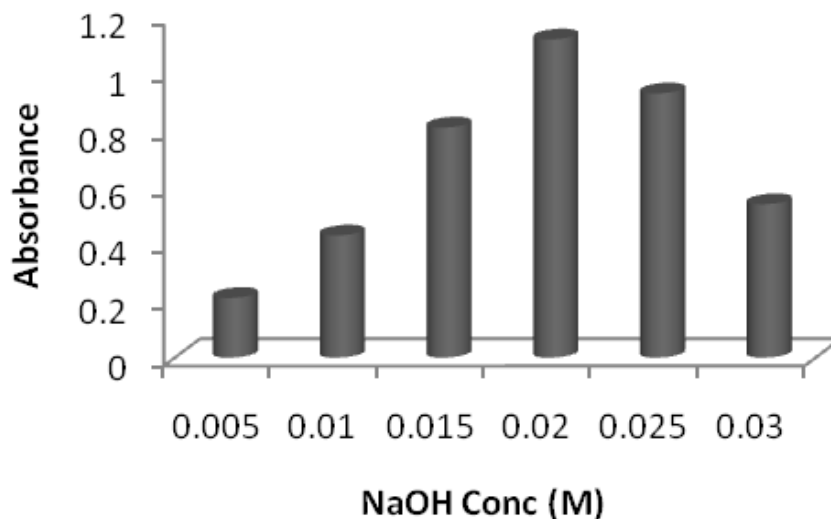
**Figure 2. Optimization of suitable wavelength for spectrophotometric determination of pendimethalin.**

### **Application of the spectrophotometric method**

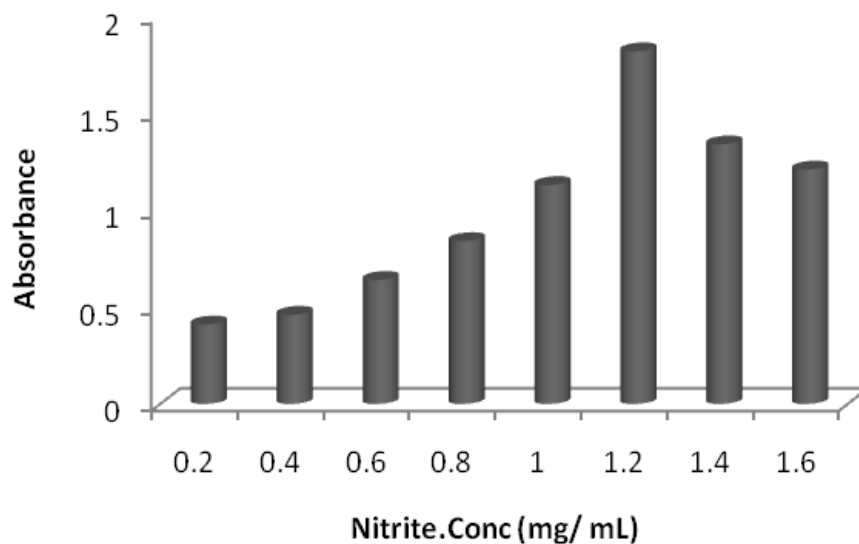
#### **% Recovery**

Samples of soil, water and potato were collected from crop field where no spray of the herbicide was done. Control samples (10 g) of each were separately placed in bottles and known concentration of pendimethalin solution was added to adjust the concentration level of 5, 10, 15  $\mu\text{g g}^{-1}$ . The samples were homogenized by mechanical shaking for uniform mixing and kept for 6 hours at ambient temperature. The homogenized samples were extracted for 60 min with 25 mL of each, methanol and acetone. Extraction was repeated

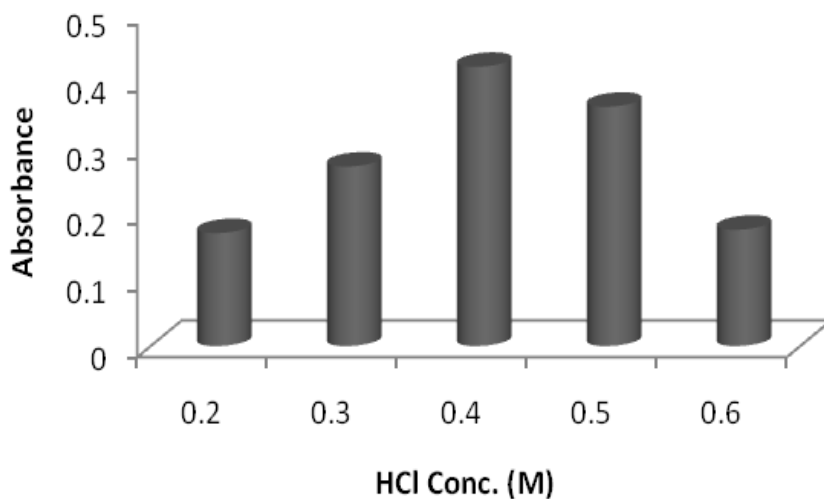
three times. The percent recovery was evaluated by the proposed method, using both batch and flow injection analysis. Each recovery was carried out in triplicate (Table-2).



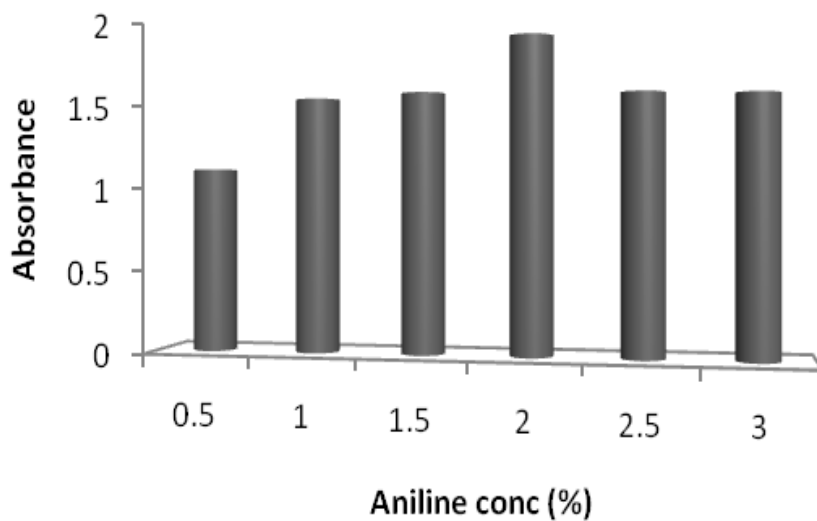
**Figure 3. Optimization of NaOH solution (2M) volume for spectrophotometric determination of pendimethalin.**



**Figure 4. Optimization of nitrite solution ( $1000 \mu\text{g mL}^{-1}$ ) volume for diazotization of hydrolyzed pendimethalin.**



**Figure 5. Optimization of HCl solution concentration for diazotization of hydrolyzed pendimethalin.**



**Figure 6. Optimization of aniline concentration for coupling of diazotized Pendimethalin.**



### Determination of residue in vegetables samples

The pendimethalin residue in cabbage, potato, onion and garlic sample collected from a crop field, (Peshawar and Swat) upon which the herbicide was sprayed as a pilot project, was determined by standard addition method. Six samples of vegetables (10 g each) were taken in bottles and spiked with a known concentration of standard pendimethalin solution. The bottles were uniformly shaken manually for proper mixing and kept for 24 hours. The herbicide was extracted with 25 mL of solvent (acetone and methanol). The extract of acetone was evaporated at room temperature and the residue was dissolved in 25 mL methanol. The concentrations of extracts were then evaluated by the proposed spectrophotometric method (Tadeo *et al.*, 2000). The residue of herbicide present in the sample was determined from the difference of the concentration of added standard solution and the concentration of herbicide in spiked sample (Table-3).

### Determination in commercial formulation

Sample (pendimethalin) was purchased from local market. An amount of 1-3 mL of pendimethalin was taken in titration flask and hydrolyzed in the same way as standards. From these hydrolyzed solutions 2 mL were taken in 50 mL volumetric flasks and color was developed in the same way as mentioned before. The absorbance of the colored solution was measured at 460 nm. The results in formulation are given in (Table-4).

### CONCLUSION

Spectrophotometric method is presented for determination of dinitroaniline herbicides in food samples and formulations using diazotization method. The method is sensitive, simple and suitable in routine laboratories for application to environmental samples and formulation. Compared with chromatographic methods, the present method is cheaper, simpler and does not require as much purification of sample as required for chromatographic analysis.

**Table-1. Optical characteristics of the proposed method.**

Parameter	Value
$\lambda$ max (nm)	460
Molar absorptivity ( $\epsilon$ ) L mol <sup>-1</sup> cm <sup>-1</sup>	$1.5 \times 10^4$
Standard Deviation (S)	0.069
RSD (%)	13.7
Limit of Detection ( $\mu\text{g mL}^{-1}$ )	0.208
Limit of Quantification ( $\mu\text{g mL}^{-1}$ )	0.69
Correlation Coefficient	0.997
Slope	0.0116
Intercept	0.0027

**Table-2. Application of the method for the determination of dinitroaniline herbicides.**

S.No	Spiked Sample	Added ( $\mu\text{g}$ )	Found ( $\mu\text{g}$ )	Average % Recovery	
			Proposed method		
1	Soil	500	400	80 $\pm$ 0.38	
		1000	900	90 $\pm$ 0.41	
		1500	1200	80 $\pm$ 0.21	
2	Potato	500	400	80 $\pm$ 0.38	
		1000	900	90 $\pm$ 0.41	
		1500	1200	80 $\pm$ 0.21	
3	Water	500	450	90 $\pm$ 0.37	
		1000	1050	105 $\pm$ 0.27	
		1500	1600	106 $\pm$ 0.15	

**Table-3. Residue level of pendimethalin from real sample.**

S.No.	Samples	Residue $\mu\text{g g}^{-1}$
1.	Garlic	1.37 $\pm$ 1.24
2.	Water	1.11 $\pm$ 2.18
3.	Cabbage	1.41 $\pm$ 1.26
4.	Potato	1.32 $\pm$ 1.18

**Table-4. Concentration of pendimethalin in commercial formulation.**

Commercial formulation	Labeled Amount ( $\text{g mL}^{-1}$ )	Proposed Method ( $\text{g mL}^{-1}$ )
Active ingredient	0.82 $\pm$ 0.46	0.76 $\pm$ 0.38

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