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Dissipation Study of Mepiquat Chloride and its Metabolites in Water at Different pH Levels

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Abstract : The dissipation behavior of mepiquat chloride in acidic, neutral and basic water was studied using mepiquat chloride 25% SL formulation. The formulation was spiked in different aqueous buffer solutions at different concentrations, such as T0 – Untreated Control, T1 – mepiquat chloride 1.0 mg/L and T2 – mepiquat chloride 2.0 mg/L. The spiked samples were exposed to sun light and collected at different pre-determined sampling occasions such as 0, 1, 3, 5, 7, 10, 15 and 20 days for acidic water, neutral and basic water. These collected samples were analyzed until the residues were below determination level in each of the buffer solution. The content of mepiquat chloride was analyzed by a validated UV-VIS Spectro-photometric method and its metabolites N-methyl piperidine and piperidine were analyzed using validated GC-FID method. The related dissipation data was obtained.

Key Words : Mepiquat Chloride, N-methyl piperidine, Piperidine, Dissipation, DT50 and aqueous buffer solutions.

Introduction

Pesticides are valuable for elevated agricultural production. The secure use of the pesticide relies upon its toxicological properties and its distribution and persistence within the environment, along with consideration of any unusual photoproducts and metabolites that are probably formed[1]. Fallacious utilization of pesticides ends in environmental contamination[2]. There has been increased research interest aimed at understanding the process that controls the penetration of pesticides into the soil and their subsequent contamination of groundwater and surface water[3]. Accumulation of these poisonous pesticides in groundwater is mainly caused by the physical, chemical, and biological processes. Thermal decomposition, changes in pH of water and hydrolysis cause the breakdown and degradation of pesticides [4,5,6,7, 8]. Water is responsible for considerable breakdown of pesticide molecules and varies extensively with pH extremes[9]. The determination of pesticide dissipation from water is a necessary requirement for pesticide registration in Australia, and data are readily available for standard conditions. Most of the time, first-order dissipation half-life is determined from distilled or tap water. Sometimes the half-life is additionally separated into mechanistic removal rates like photolysis, chemical reaction and volatilization[10]. Although this gives an idea of the relative longevity of different chemicals, the procedure may not accurately describe a pesticide's persistence in natural waters, for example, river, ground or marine water. This is because dissipation varies with the pesticide and the temperature,

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reduction potential, chemistry and biological properties of the water. At times, different removal processes may dominate, causing the dissipation to exhibit bi-phasic or tri-phasic features. Consequently, pesticide half-lives are often presented as a range of values[11].

Mepiquat chloride, 1,1-Dimethylpiperidinium chloride, is greatly used for plant growth as mixtures, or along with other pesticides[12]. Mepiquat chloride is a quaternary ammonium pesticide which is soluble in water, non-volatile, thermally stable, stable in acid and quite steady to hydrolytic or biological degradation.

The present research was targeted to investigate the fate of residues of mepiquat chloride and its metabolites (N-methyl piperidine and Piperidine) in three different aqueous buffer solutions under sun light.

Materials and Methods

Reference analytical standards of mepiquat chloride (purity 99.1%), N-methyl piperidine (purity 98.3%), Piperidine (purity 99.8%) were obtained from Sigma Aldrich. The test item mepiquat chloride 25 SL (Soluble Concentrate) formulation was purchased from local market. Acetonitrile, Water HPLC grade, Methanol HPLC grade, 1,2-Dichloroethane LR grade, Ferric chloride AR grade, Hydrochloric acid AR grade, Ferric chloride GR grade and Formic acid AR grade were obtained from the Merck India limited. Distilled water was purified by using the milli-Q Plus apparatus (Millipore, Bedford, MA, USA).

Preparation of mepiquat chloride and its metabolite reference standard stock solutions

Accurately 5.23 mg of reference analytical standard of mepiquat chloride was weighed in to 10mL volumetric flask. The content was dissolved and the volume was brought up to the mark using methanol. This stock solution represents 518 mg/L of mepiquat chloride.

N-methyl piperidine and Piperidine standard stock solutions

Accurately weighed 5.82 and 5.75 mg of metabolites reference analytical standard, N-methyl piperidine and piperidine in to 10 mL volumetric flask. The content of the flask was dissolved and the volume was brought up to the mark using methanol.

Preparation of calibration solutions mepiquat chloride

A series of calibration solutions (0.05 – 5 mg/L) were prepared in 25 mL volumetric flask by diluting appropriate volumes of working standard solution and it was made up to the mark using methanol. A calibration curve has been plotted for concentration of the standards versus absorbance at 364 nm and the linearity of the method was determined from the correlation coefficient. Calibration was performed at every step.

Metabolites N-methyl piperidine and Piperidine

Mixture of different known concentrations of metabolites N-methyl piperidine and Piperidine (0.02 – 2 mg/L) were prepared in methanol by diluting the stock solution. Injected the standard solutions and measured the peak area. A standard calibration curve has been premeditated for concentration of the standards injected versus area obtained and the linearity of the method was determined from the correlation coefficient.

Sample stock solution

Accurately 199.44 mg of test item (purity 25.07%) of mepiquat chloride was taken into a 50 mL volumetric flask. The content was dissolved in 10 mL of acetonitrile, sonicated and made up to the mark with acetonitrile. The concentration was 1000 mg/L solution. The stock sample solution was used for preparation of dose samples (T1 and T2) in different aqueous buffers.

Preparation of acidic water (pH 4.0)

Accurately 8.11 g of potassium dihydrogen phosphate was dissolved in 300 mL of distilled water and diluted to 1000 mL. The pH was adjusted to 4.0 with the same.

Preparation of neutral water (pH 7.0)

Accurately 6.33 g of disodium hydrogen phosphate and 5 g of potassium dihydrogen phosphate were dissolved in 300 mL of distilled water and diluted to 1000 mL. The pH of the buffer was adjusted with disodium hydrogen ortho phosphate and potassium dihydrogen phosphate.

Preparation of basic water (pH 9.0)

Accurately 12.52 g of boric acid & 15.07 g of potassium chloride were dissolved in 300 mL of distilled water and diluted to 1000 mL. The pH was adjusted to 9.0 using 0.1 M sodium hydroxide solution.

Expermental

Spectrophotometric parameters for the determination of mepiquat chloride:

Instrument	:	Shimadzu spectrophotometer UV 1601 with UV 601 software system
Cuvette	:	1 cm^2 quartz cell
Measuring mode	:	Absorbance
Wavelength range Lambda max	:	330 – 430 nm
	:	364 nm

Gas chromatograph Instrument Conditions for Mepiquat chloride Metabolites – (N-methyl Piperidine & Piperidine):

Instrument	:	Shimadzu Gas Chromatographic with flame ionization detector (FID) auto injector with GC Solution Software
Column used	:	DB-17 (30 m length x 0.53 mm x 1 µm film thickness)
Detector	:	Flame Ionization Detector
Gas flow rate	:	Make up Nitrogen (N2):30 mL/min Hydrogen:40 mL/min Air:400 mL/min
Column Flow Rate	:	4.0 mL/min
Temperature conditions	:	Injector:195°C Detector:215°C
Column oven	:	Initially maintained at 50°Cfor 2 min, then increased @ 30°C/min. up to 150°C and hold for 5 min.
Injection Volume	:	1.0 µL
Solvent	:	Methanol
Run time	:	10 min
Retention time (approximate)	:	Piperidine: 3.8 min N-methyl Piperidine: 4.8 min

Derivation procedure for mepiquat chloride and its Metabolites in water samples

To the 25 mL of respective water sample (acidic, neutral and basic), 10 mL of 7 N HCl and 5 mL of 30% ferric chloride solution in 7 N HCl were added and mixed well for 5 minutes. 25 ml of 1, 2-dichloroethane was added and then extracted the ion pair into the organic phase by shaking the flask for 20 min. Allowed the phases to separate. The organic layer was collected. This step was repeated twice with 25 ml portion of 1, 2-dichloroethane and concentrated the combined organic layer to 25 ml after filtering through Whatman filter paper. Measured the absorbance at 364 nm against a blank using UV-VIS spectrophotometer. Residue of N-methyl piperidine and piperidine residues were determined by GC-FID.

Dissipation kinetics Study

The control (T0) and spiked (T1 and T2) samples were stored under sun light and collected on pre-determined sampling occasions such as 0, 1, 3, 5, 7, 10, 15 and 20 days for acidic water (pH 4), neutral (pH 7) and basic water (pH 9). The collected samples were analyzed for mepiquat chloride and its metabolites (N-methyl piperidine and Piperidine). All the samples were analyzed until the residues were at below determination level in each of the buffer solution. Residues of mepiquat chloride were analyzed using the validated UV-VIS Spectro- photometric method and its metabolites N-methyl piperidine and piperidine by a validated GC-FID method.

Application data

Name of the buffers	Acidic, Neutral and Basic
Replications	Three
Method of fortification	T_0 (Untreated Control) T1 (1 mg/L Level) 1.0 mL of test item stock solution was fortified in to 1000 mL (1Liter) different buffer samples to get the uniform concentrations. T2 (2 mg/L Level) 2.0 mL of test item stock solution was fortified in to 1000 mL (1Liter) different buffer samples to get the uniform concentrations.

Sampling data

Samples Exposure	Under direct sunlight	
Sample preparation	During each sampling occasion, water samples were mixed thoroughly and sub sampled 20 mL using a pipette.	
Buffers (Occasions)	pH 4, pH 7, pH 9 (0, 1, 3, 5, 7, 10 15 and 20 days)	
Laboratory condition	**Temperature**	
	Minimum	Maximum
	20.3°C	25.2°C

Method validation

Method validation ensures the analysis credibility. In this study, the parameters accuracy, precision, linearity, Limits of Detection and Quantification were considered (Nageswara Rao et al. 2016). The accuracy of the method was determined by recovery tests using samples spiked at concentration levels 0.1, 1.0 µg/mL of mepiquat chloride and 0.05, 0.5 µg/mL levels of N-methyl piperidine and piperidine. Linearity was determined by different known concentrations of mepiquat chloride (0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/mL) and N-methyl piperidine and piperidine (0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) which were prepared by diluting the stock solutions. The signal to noise ratio 3:1 was considered for calculating the limit of detection. The signal to noise ratio 10:1 was considered for calculating the limit of quantification (SANCO Guidelines, 2009). The limit of quantification (LOQ) for mepiquat chloride was 0.1 mg/L and for metabolites N-methyl piperidine and Piperidine the (LOQ) was 0.05 mg/L.

Results and Discussion

Linearity

Mepiquat chloride and its metabolites

The method was found to be linear with a correlation coefficient of 0.9998 for mepiquat chloride (0.1 – 10 mg/L), 0.9998 for metabolite Piperidine and 0.9995 for metabolite N-methyl piperidine when tested in the range of 0.05 – 5 mg/L. Calibration curves were presented in **Figure. 1** and **Figure. 2.**

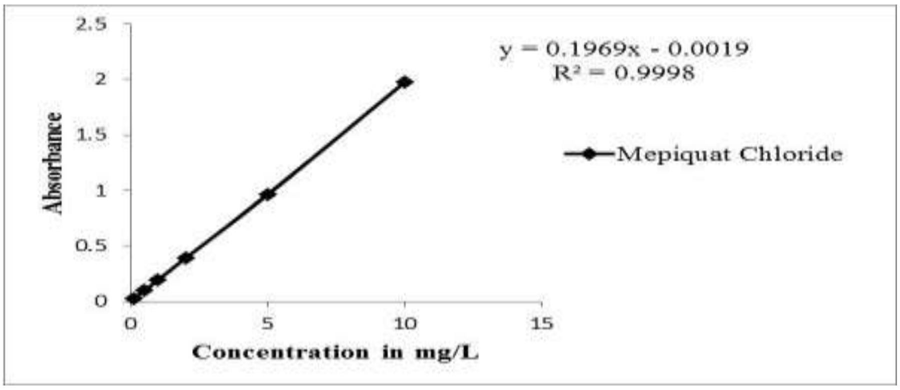

Figure 1. calibration curve of mepiquat chloride

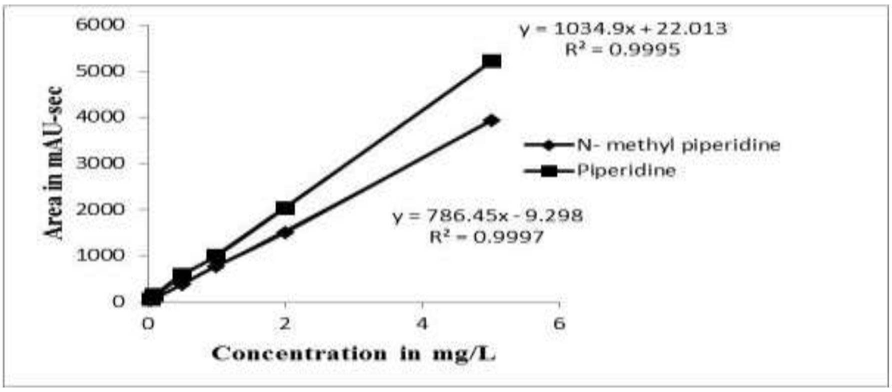

Figure 2. Calibration curve of N-methyl piperidine and Piperidine

Recovery

The average percent recovery for mepiquat chloride in acidic, neutral and basic water was 88 ± 2.36; 89 ± 3.12 and 86 ± 2.89 at 0.1 mg/L fortification level, 91 ± 1.36; 93 ± 2.02 and 90 ± 2.78 at 1.0 mg/L fortification level respectively. The average percent recovery of metabolite N-methyl piperidine content in acidic, neutral and basic water was 88 ± 2.04; 86 ± 2.37 and 87 ± 2.86 at 0.05 mg/L fortification level, 93 ± 1.87; 91 ± 1.17 and 90 ± 1.91 at 0.5 mg/L fortification level respectively. The average percent recovery of metabolite Piperidine in acidic, neutral and basic water was 88 ± 2.48; 85 ± 2.29 and 86 ± 2.77 at 0.05 mg/L fortification level, 92 ± 1.77; 91 ± 1.42 and 89 ± 1.49 at 0.5 mg/L fortification level.

Limit of Quantification (LOQ)

The limit of Quantification (LOQ) for mepiquat chloride was 0.1 mg/L. The LOQ for metabolites of mepiquat chloride viz., N-methyl piperidine and piperidine were 0.05 mg/L.

Limit of Detection (LOD)

The limit of Detection (LOD) for mepiquat chloride was 0.03 mg/L. The LOD for metabolites of mepiquat chloride viz., N-methyl piperidine and piperidine were 0.02 mg/L.

Dissipation details

Acidic water

Mepiquat chloride

Analysis of acidic water samples collected on ‘0’ day showed 0.925 mg/L and 1.929 mg/L in T1 and T2 tested dosages respectively. The 1st day samples showed 0.718 mg/L and 1.475 mg/L at T1 and T2 dosages. The 3rd day samples showed 0.495 mg/L and 0.998 mg/L at T1 and T2 dosages, respectively. On 5th day, residues have dissipated to 0.428 mg/L in T1 and 0.759 mg/L in T2. Analysis of residues on 7th day showed 0.331 mg/L in T1 and 0.621 mg/L in T2 tested dosages. By 10th day the residue was found to be 0.274 mg/L and 0.563 mg/L and on 15th day it was 0.158 mg/L and 0.347 mg/L in T1 and T2 tested dosages. Complete dissipation of residues was observed by 20th day.

Piperidine

Analysis of acidic water samples collected on ‘0’ day, 1st day and 3rd day showed BDL in T1 and T2 tested dosages respectively. The 5th day samples showed 0.089 mg/L and 0.191 mg/L in T1 and T2 dosages, respectively. On 7th day residues have dissipated to below determination level for T1 dosage and 0.101 mg/L for T2 tested dosage. Complete dissipation of residues was observed on 10th day, 15th day and 20th day in both the tested dosages T1 and T2.

N-methyl piperidine

Analysis of acidic water samples collected on ‘0’ day, 1st day and 3rd day showed BDL in T1 and T2 tested dosages respectively. The 5th day samples showed 0.131 mg/L and 0.251 mg/L for T1 and T2 dosages, respectively. On 7th day residues have dissipated to below determination level in T1 dosage and 0.093 mg/L in T2 tested dosage. Complete dissipation of residues was observed on 10th day, 15th day and 20th day in both the tested dosages T1 and T2.

Neutral water

Mepiquat chloride

Analysis of neutral water samples were collected on ‘0’ day showed 0.917 mg/L and 1.839 mg/L in T1 and T2 tested dosages respectively. The 1st day samples showed 0.628 mg/L and 1.276 mg/L in T1 and T2 dosages. The 3rd day samples showed 0.487 mg/L and 0.969 mg/L in T1 and T2 dosages, respectively. On 5th day residues had dissipated to 0.401 mg/L in T1 and 0.867 mg/L in T2. Analysis of residues on 7th day showed 0.301 mg/L in T1 and 0.635 mg/L in T2 tested dosages. By 10th day the residue was found to be 0.248 mg/L and 0.446 mg/L and on 15th day it was 0.156 mg/L and 0.332 mg/L in T1 and T2 tested dosages. Complete dissipation of residues was observed on 20th day.

Piperidine

Analysis of neutral water samples collected on ‘0’ day, 1st day and 3rd day showed BDL in T1 and T2 tested dosages respectively. The 5th day samples showed 0.093 mg/L and 0.172 mg/L in T1 and T2 dosages, respectively. On 7th day residues have dissipated to below determination level in T1 dosage and 0.078 mg/L in T2 tested dosage. A complete dissipation of residues was observed on 10th day, 15th day and 20th day in both the tested dosages T1 and T2.

N-methyl piperidine

Analysis of neutral water samples collected on ‘0’ day, 1st day and 3rd day showed BDL in T1 and T2 tested dosages respectively. The 5th day samples showed 0.105 mg/L and 0.189 mg/L in T1 and T2 dosages, respectively. On 7th day residues have dissipated to below determination level in T1 dosage and 0.093 mg/L in T2 tested dosage. A complete dissipation of residues was observed on 10th day, 15th day and 20th day in both the tested dosages T1 and T2.

Basic Water

Mepiquat chloride

Analysis of basic water samples were collected on ‘0’ day showed 0.948 mg/L and 1.789 mg/L in T1 and T2 tested dosages respectively. The 1st day samples showed 0.686 mg/L and 1.313 mg/L in T1 and T2 dosages. The 3rd day samples showed 0.474 mg/L and 0.964 mg/L in T1 and T2 dosages, respectively. On 5th day residues have dissipated to 0.359 mg/L in T1 and 0.833 mg/L in T2. Analysis of residues on 7th day showed 0.314 mg/L in T1 and 0.625 mg/L in T2 tested dosages. By 10th day the residue was found to be 0.236 mg/L and 0.438 mg/L and on 15th day it was 0.136 mg/L and 0.295 mg/L in T1 and T2 tested dosages. Complete dissipation of residues was observed on 20th day.

Piperidine

Analysis of basic water samples collected on '0' day, 1st day and 3rd day showed BDL in T1 and T2 tested dosages respectively. The 5th day samples showed 0.082 mg/L and 0.197 mg/L in T1 and T2 dosages, respectively. On 7th day residues have dissipated to below determination level in T1 dosage and 0.073 mg/L in T2 tested dosage. A complete dissipation of residues was observed on 10th day, 15th day and 20th day in both the tested dosages T1 and T2.

N-methyl piperidine

Analysis of basic water samples collected on '0' day, 1st day and 3rd day showed BDL in T1 and T2 tested dosages respectively. The 5th day samples showed 0.103 mg/L and 0.215 mg/L in T1 and T2 dosages, respectively. On 7th day residues have dissipated to below determination level in T1 dosage and 0.087 mg/L in T2 tested dosage. A complete dissipation of residues was noted on 10th day, 15th day and 20th day in both the tested dosages T1 and T2.

The dissipation curve plotted between concentration of the analyte and sampling occasions is presented in **Figure. 3, Figure. 4** and **Figure. 5**. DT50 value was calculated using the following formula:

DT50 = ln 2/ (k)

where, 'k' is slope of the curve obtained from the dissipation data.

The analysis changed into carryout in laboratory circumstance at 25°C and shown the half-life values around 6.47 days in acidic water. In neutral water, the half-life was around 6.33 days. Further the degradation of pesticide in basic water was rapid; the half-life value was around 5.24 days.

The calculated DT 50 (Time required to degrade 50% of residues) values of mepiquat chloride in different pH waters (Acidic (pH 4), Neutral (pH 7) and Basic (pH 9)) under the influence of sunlight were presented in **Table 1.** The rate constant value was calculated by linear regression equation from the first order rate equation:

K = ln a/a-x/dt

where, dt is the time interval between t_1 and t_2 and a, x is the concentration of pesticides at times t_1 and t_2 respectively. A plot of concentration of the residues and rate with the R^2 indicates first order kinetics in dissipation of the fungicide. The DT50 (Half Life) of mepiquat chloride calculated by regression analysis from the dissipation data.

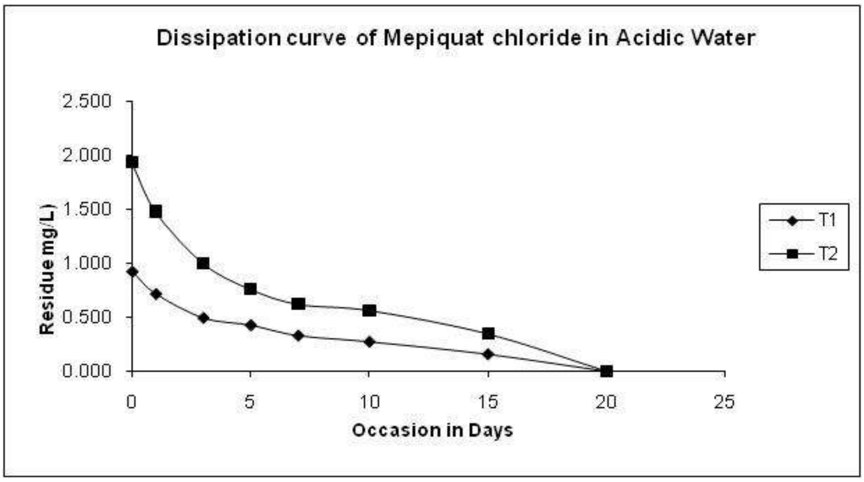

Figure.3. Dissipation curve of mepiquat chloride in acidic water

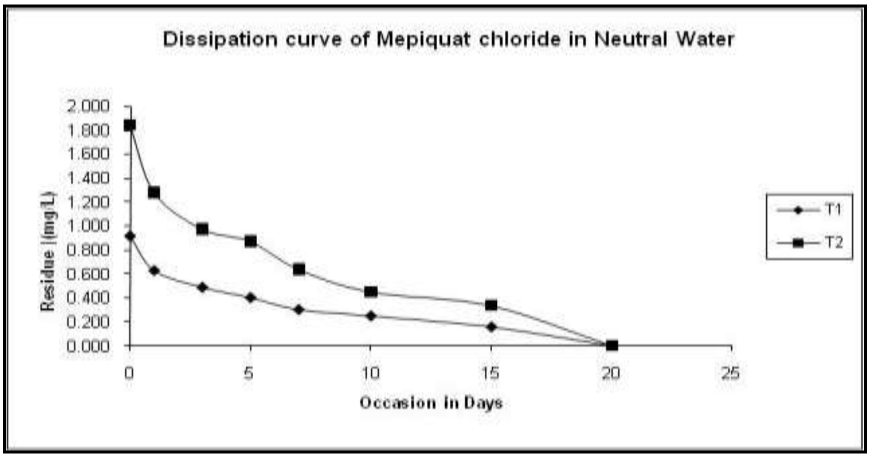

Figure. 4. Dissipation curve of mepiquat chloride in neutral water

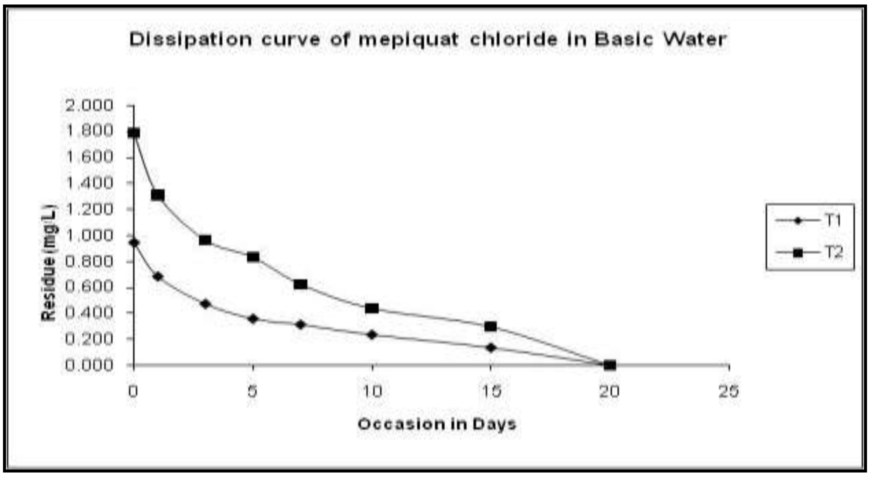

Figure. 5. Dissipation curve of mepiquat chloride in basic water

Table 1. **Regression Analysis of mepiquat chloride**

Content	**Residue Level (T1)**			**Residue Level (T2)**		
	pH 4	**pH 7**	**pH 9**	**pH 4**	**pH 7**	**pH 9**
Slope	-0.048	-0.048	-0.058	-0.047	-0.048	-0.057
Half-life	6.26	6.32	5.22	6.47	6.33	5.24
Intercept	-0.103	-0.133	-0.098	0.189	0.174	0.201
CC	0.986	0.979	0.971	0.966	0.977	0.988

Conclusion

The dissipation kinetics of mepiquat chloride in different pH waters under direct sunlight was quicker whilst compared with the dissipation facts acquired at 25°C. In basic water degradation was faster while compared to acidic and neutral water. Dissipation of mepiquat chloride in different pH waters followed first order kinetics and this review describes a fast, simple sensitive analytical method based on UV-VIS spectrophotometry to determine the mepiquat chloride residues in three different types of buffers. GC-FID was used for the excellent separation of N-methyl piperidine and Piperidine in three different types of buffers in short run time (around 10 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision, LOQ and DT50 values were established by following South African National Civic Organization (SANCO) and Environmental Protection

Agency (EPA) guidelines. Hence the proposed analytical procedure and dissipation information would be valuable for regulatory monitoring authority, residue labs and research scholars to determine the mepiquat chloride residues in different commodities (crop, water and soil samples).

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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