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A New Validated Method for the Determination of Dodine by HPLC

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ABSTRACT

The present work reports a simple and rapid reversed-phase high-performance liquid chromatography (RP-HPLC) method for the determination of dodine. A strategic experimental approach was implemented for the method development. The desired chromatographic conditions was achieved on a Phenomenex C8 (4.6×250 mm, 5 µm) column using isocratic elution. The optimized mobile phase is a mixture of aqueous and organic modifier (Acetonitrile) containing 0.15% (v/v) trifluoroacetic acid. Dodine was monitored at 205 nm wavelength using UV detector. The method is effective for the determination of dodine quantitatively. Calibration curve was linear and regression coefficient was found to be 0.999 at a concentration of 8.45 to 84.47 mg/L. The % RSD of precision of method was found 0.13% and accuracy found between 98.68% - 100.60%. The proposed method was successfully applied for the determination of dodine in technical.

KEYWORDS

Introduction

Dodine is a fungicide and bactericide used to control scab on apples, pears and pecans, brown rot on peaches and several foliar diseases of cherries, strawberries, peaches, sycamore trees and black walnuts. It is also used as an industrial biocide and preservative. The compound works by changing the cell walls of the fungus, causing loss of the materials from within the cell. It is available[1] as a soluble concentrate (SC) and a wettable powder (WP). Dodine is determined by non-aqueous titrimetric method[2,3] (IS 13784/85: 1993) but the raw material and process impurities formed will also interfere because same method is used for their purity assignment. A GC-MS detection derivatization method[4,5] with hexafluoroacetylacetone is also available but it requires 2 hrs for the derivatization which is time consuming. The other method[6] for determination of dodine by ion-pair HPLC-UV is available which is MS non compatible mobile phase and require column cleaning procedure. The aim of this work was the development of a simple RP-HPLC with direct UV-detection method for the determination of Dodine. The developed method was validated according to SANCO[7] and CIPAC[8] guidelines to show the capability of the method.

Methods

Materials: Dodine standard was purchased from Sigma Aldrich. HPLC grade solvents were used for mobile phase and sample preparation. Syringe filters were purchased from J-Sil. Dodine technical was taken from Indofil Industries Limited.

Chromatographic conditions: The chromatographic conditions was optimized[9] using the Phenomenex C8, 5 µm (250 mm×4.6 mm) column. The optimised mobile phase is a 0.15% (v/v) of trifluoroacetic acid in solvent A (Acetonitrile) and solvent B (HPLC Water). Both the solvents were filtered through a 0.22 µm PVDF (Hydrophilic polyvinylidene fluoride) membrane filter before mixing and degassed by sonication prior to use. Method is an isocratic elution using mixture of solvent A and B in 60:40 proportions. Mobile phase was used as diluent. The final selected and optimized conditions were as follows;

Instrument	:	HPLC, Shimadzu, LC-20AD with PDA detector
Mobile Phase	:	60:40 (A:B) 0.15% (v/v) Trifluoroacetic acid
		Solvent A-Acetonitrile
		Solvent B-Water containing
Column	:	Phenomenex C8, 250 mm x 4.6 mm x 5.0 µ
Flow rate (mL/minute)	:	1.0
Column Oven Temperature (° C)	:	30
Injection volume (µL)	:	20
Wavelength (nm)	:	205
Elution Program	:	Isocratic
Run Time (minutes)	:	20.0

Preparations

Standard Preparation: For the determination of dodine, the standard solution was prepared by dissolving and diluting 65 mg dodine with mobile phase into the volumetric flask of 25 mL and the volume was made up to the mark with the mobile phase, then 1.0 mL was taken from the above and was further diluted with the mobile phase up to 50 mL to produce the working standard concentration of 0.0506 mg/mL.

Sample preparation: For the determination of dodine, the sample solution was prepared by dissolving and diluting 65 mg dodine with mobile phase into the volumetric flask of 25 mL and the volume was made up to the mark with the mobile phase, then 1.0 mL was taken from the above and was further diluted with the mobile phase up to 50 mL and applied to HPLC system for the analysis.

$$\text{Dodine content, \% w/w} = \frac{M_1 \times A_2 \times P}{M_2 \times A_1}$$

M_1 = Weight of standard in mg for standard solution preparation.

M_2 = Weight of sample taken in mg for the test solution preparation.
A_1 = Mean Peak Area of Dodine in standard chromatogram
A_2 = Mean Peak Area of Dodine in sample chromatogram
P = Percent purity of Dodine reference standard

Results and Discussion
Method validation[10]

System Suitability: From chromatogram of the standard preparation (six replicate injections), measured the peak area responses for the analyte peak and evaluated the system suitability parameters **(Table 1)**.

Acceptance criteria: %RSD for replicate injections of peak area response of peak from the standard preparation should be not more than 2.0.

The Tailing factor of peak should be not more than 2.0.

Theoretical plate of peak should be not less than 2000.

Table 1: System suitability data for Dodine

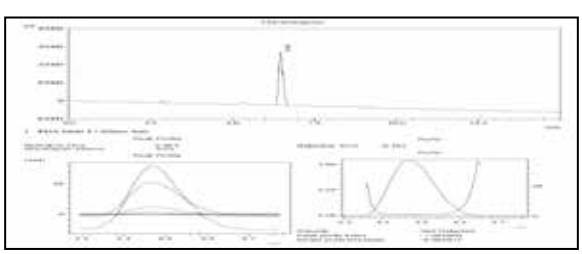
Compound	RT	Theoretical Plates	Tailing Factor
Dodine Standard	6.46	10543	1.34
Dodine Technical	6.46	10432	1.34

Specificity and Selectivity: Specificity is the ability of the method to measure the analyte response in the presence of diluent. **Figures 1** and **2** show that there is no interference at the RT (Retention Time) due to the blank and **Table 2** shows the summery peak purity results to assess the ability of the method. Therefore, the specificity of the method was judged from the absence from the interfering peaks (false peaks) at the analyte elution times from blank chromatograms.

Acceptance criteria:
No interference at the retention time of dodine from diluent.

Table 2: System Suitability for Dodine

Compound	RT	Peak purity index	Impurity/ Interference
Dodine Standard	6.46	1.0000	Not detected
Dodine Technical	6.46	0.9998	Not detected



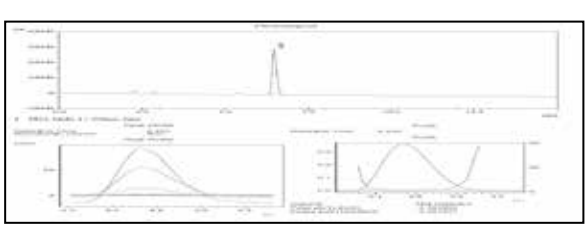


Figure 1: Peak Purity Chromatogram of Dodine Standard
Figure 2: Peak Purity Chromatogram of Dodine technical

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of the analyte in a sample within a given range. The response function was determined by preparing standard solutions at nine different concentration levels ranging from 8.45 mg/L to 84.47 mg/L. The response was found to be linear for the specified concentration of the standard. The regression coefficient was 0.999. The linearity concentration and the regression statistics are shown in **Table 3** and Linearity curve is presented in **Figure 3**.

Acceptance criteria:
Regression coefficient (r2) > 0.999

Y-intercept (close to zero), slope of regression line should be reported.

Table 3: Linearity data of dodine standard

Concentration (mg/L)	Mean Peak Area Counts
8.45	42434
16.89	86114
25.34	128512
33.79	172465
50.68	262453
59.13	298481
67.57	338166
76.02	380925
84.47	432900

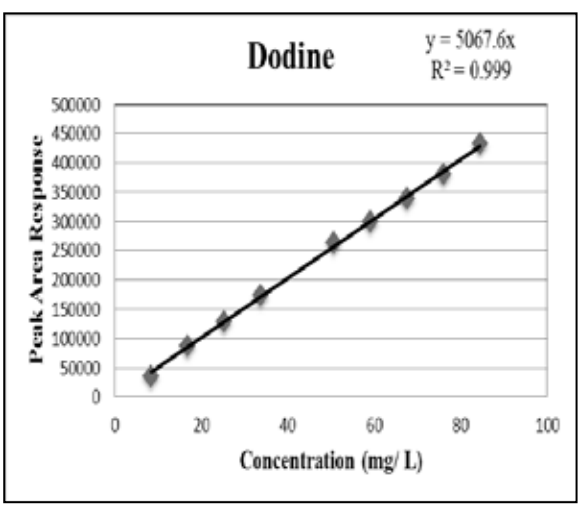


Figure 3:- Linearity curve for dodine standard

Intercept with y-axis (x)	0.000
Slope (m)	5067.6
Regression Coefficient (r2)	0.999

LOD and LOQ: Signal-to-noise ratios of > 3:1 and > 10:1 was obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 0.63 mg/L and 2.10 mg/mL for Dodine. The % RSD of peak areas at quantification limit was achieved by injecting seven replications was well within the acceptance limit (not more than 2.2 %, as per Horwitz equation). The determined lower limit of detection, lower limit of quantification and precision at LOQ values for all components are presented in **Table 4.**

Acceptance criteria:

S/N ration should be > 3.0
S/N ration should be > 10.0

Table 4: Limit of detection and limit of quantification

Component Name	LOD		S/N Ratio	LOQ		S/N Ratio
	mg/ mL	%		mg/ mL	%	
Dodine	0.63	1.21	4.16	2.1	4.04	12.53

Precision: Precision (Repeatability) of analytical method was determined by analyzing seven sample solutions and assayed for active ingredient content in each replicate of test item. The % RSD of active ingredient content was 0.13%. This indicates that the system is precise and suitable for determination dodine. The results obtained are shown in **Table 5**. The parameters all complied with the acceptance criteria and system suitability was established.

Acceptance criteria:
% RSD of active content should not be more than 2.0.

Table 5: Precision (Repeatability) data for dodine technical.

Sample No	Active content (%, w/w)	Mean Content
1	98.53	98.63
	98.74	
2	98.91	98.78
	98.61	
3	98.95	98.8
	98.65	
4	98.88	98.63
	98.39	
5	98.13	98.49
	98.85	
6	98.47	98.47
	98.46	
7	98.90	98.65
	98.39	
Mean		98.64
Standard Deviation		0.1270
% RSD		0.13

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. To confirm the accuracy of the proposed method, recovery experiments were carried out by the standard addition technique. The accuracy of the method was carried out by adding known amounts of three concentration levels; 0.5%, 1.0%, and 1.5% of the target concentration to the sample examined. The samples were prepared as per the described procedure. The percentage recoveries of all components at each level were determined. The mean of percentage recoveries (n=3) and the relative standard deviation were calculated. The mean recovery was found 99.88% (from 98.68% to 100.60%), which is within the limit indicates that the proposed method is accurate (**Table 6**).

Acceptance criteria:
The percentage recovery should be in the range of 98.0% to 102%.

Table 6: Accuracy (%Recovery) data for dodine technical.

Level. No.	% Level	mg		Recovery (%, w/w)
		Added	Found	
I	0.5	0.3215	0.3227	100.37
II	1.0	0.6430	0.6345	98.68
III	1.5	0.9645	o.9703	100.60

Sample Results: The experimental results of the amount of Dodine in its technical, expressed as a percentage of label claim were in good agreement[11] with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. Five different batches of Dodine technical were analyzed using the proposed procedures. The active ingredient content was found for Dodine as shown in **Table 7**.

Table 7: Active content results in Dodine technical.

Batch No.	Active content (%, w/w)	Mean Content
1	98.67	98.60
	98.54	
2	98.73	98.81
	98.89	
3	98.55	98.72
	98.89	
4	98.67	98.82
	98.97	
5	98.59	98.63
	98.66	

Conclusion: A simple reverse phase HPLC method was successfully developed for the estimation of Dodine in the technical. The method validation results proved that the method is specific, linear, precise and accurate.

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