

Development and Validation of Single Laboratory Ultra Fast Liquid Chromatography Method for Quantification of Buprofezin in Suspension Concentrate (SC) Formulation

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ABSTRACT

A novel ultra fast liquid chromatography (UFLC) method has been developed and validated for quantification of Buprofezin in Suspension Concentrate (SC) formulation, using Shimadzu, packed with C18 (250 mm × 4.6 mm, 5 μm) column. Mixture of acetonitrile:water (80:20 v/v) was used as mobile phase. The flow rate was kept 1.5 ml/min and detection was carried out at 250 nm. The linearity of proposed method was investigated in the range of 200-1002 PPM ($r^2=0.999$). The percentage recovery found to be 99.87%. The % RSD value for precision study was <0.68 as per modified Horwitz equation as requirements by CIPAC. The developed method was found to be specific, linear, precise and accurate. This method is also useful for quantification of Buprofezin in their single or combination formulated products, environmental samples (soil, water), and agricultural products for pesticide residue analysis.

Keywords: Buprofezin res; Horwitz equation; Method validation, CIPAC - Collaborative International Pesticides Analytical council, Uncertainty in measurements.

I. INTRODUCTION

Buprofezin is (2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one) an insecticide and acaricide with persistent parvicidal action against Homopterasome coleoptera and acarina. Buprofezin is effective against leaf-hoppers in rice and potatoes, whitefly in citrus plants, cotton and vegetables and also for coccidae, diaspididae (scale insects) and pseudococcidae (mealybugs) in citrus plants. It is an insecticide with contact and stomach side effects.

Buprofezin is manufactured in several commercially available pesticide formulations as Suspension Concentrate (SC) in a 25% (w/w) concentration [1]. Chromatographic techniques have been the most widely used procedures for the determination of buprofezin residues at trace levels. This pesticide has been determined in fruits by high performance liquid chromatography/mass spectrometry [2], in wines by head-space gas chromatography with electron capture detection [3] and in air by gas chromatography/mass spectrometry [4]. There is only a single precedent for

the determination of buprofezin in pesticide formulations [5], which is based on pesticide extraction with chloroform and subsequent measurement in the mid-infrared region (FT-IR). It is a growth regulator, acting as an inhibitor of chitin synthesis. It is banned in some countries due to its negative environmental impacts, being especially toxic to aquatic organisms as well as non-target insects, though is of low toxicity to humans and other mammals [6, 7, 8, 9].

To the best of our knowledge, there is no reported method for quantification of Buprofezin in Suspension Concentrate (SC) formulation. Thus, efforts were made to develop fast, selective and sensitive method for quantification of Buprofezin in their Suspension Concentrate (SC) formulation using ultra fast liquid chromatographic method. In the current work developed a simple, reliable and reproducible, UFLC method which was duly validated by statistical parameters precision, accuracy-recovery, linearity. Uncertainties in measurements were also calculated for each active ingredient. The method has been applied to the estimation of Buprofezin in Suspension Concentrate (SC) formulation.

II. EXPERIMENTAL

A. Materials

A certified Reference material (CRM) of Buprofezin was procured from Sigma Aldrich. The technical grade materials of above active ingredients were obtained from market. The analytical standards were prepared by purification of these technical grade materials. The analytical standard was qualified against CRM and calculated purity found as for Buprofezin- 98.15%. This standard used for further analysis. Sample of Pesticide formulation containing Buprofezin 25% SC were procured from market. HPLC grade acetonitrile was purchased from Fischer Scientific, Mumbai (India). Mili-Q (Millipore India Pvt. Ltd) system used to obtain HPLC grade water.

B. Instrumentation

The UFLC system used to perform development and validation of this quantification method is of Shimadzu UFLC comprised of a binary solvent pump, Photo Diode array detector and auto sampler with lab solutions software.

C. Mobile phase preparation

The mobile phase consists of Mobile phase A – Acetonitrile and Mobile phase B – Water in 80:20(v/v) ratio. Mobile phase- B was filtered through a 0.45 µm nylon membrane (Millipore Pvt. Ltd, Bengaluru, India) and degassed in an ultrasonic bath.

D. Diluent preparation

Mobile phase used as diluent.

E. Standard preparation

The Standard stock solution prepared in 50 ml volumetric flask by dissolving 50 mg of Buprofezin (98.15%) standard in 10 ml of diluent. This solution then sonicated for 10 minutes and diluted to volume with diluent. This standard solution contains 1 mg/ml of Buprofezin.

F. Sample preparation

Sample solution was prepared by taking about 200 mg of Buprofezin 25% SC in 50 ml volumetric flask and about 10 ml of respective diluent was added and sonicated for 10 minutes with intermittent shaking. The content was brought back to ambient temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter.

G. Chromatographic condition

Method involves use of Shimadzu- C18 column with length of 250 mm, internal diameter 4.6 mm and 5 µm particle size of stationary phase. The column oven temperature maintained at 25°C throughout the analysis. Different compositions of mobile phase tried in isocratic mode. Mobile Phase-A: Mobile Phase-B

Acetonitrile: Water (80:20(v/v) ratio) was selected which gave good resolution. The flow rate was maintained at 1.5 ml/min and detection at 250 nm was carried out with injection volume of 20 μ l.

H. Initial analysis of sample

Sample was analyzed in accordance with section 2.5-2.7 and results were tabulated in table 1.

Calculation:

$$= \left(\frac{\text{Area of sample}}{\text{Area of standard}} \right) \times \left(\frac{\text{Weight of standard}}{\text{Weight of sample}} \right) \times (\text{purity \%}) \times (1.0)$$

Table 1. Results of initial analysis

Sr. No.	Ingredients	Active Ingredient content (A.I)
		% m/v
1.	Buprofezin	24.44

III. RESULTS AND DISCUSSION

A. Development and optimization of UFLC Method

In the present work, an analytical method based on UFLC using PDA detector has been developed and validated for the quantification of Buprofezin in Suspension Concentrate (SC) formulation. The analytical conditions were selected, keeping in mind the different chemical nature of Buprofezin.

The column selection has been done on the basis of back pressure, resolution, peak shape and day to day reproducibility of retention time. After evaluating all these factors, Shimadzu C18 (250 mm x 4.6 mm, 5 μ m particle size) column was found to be giving satisfactory results. The selection of mobile phase is based on the chemical structure of three actives. Considerably good results were obtained with water as mobile phase-B. For the selection of organic constituents of mobile phase-A, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape. Finally the mobile phase composition consisting of Mobile phase-A

(Acetonitrile): Mobile phase-B (Water) in 80:20 (v/v) ratios. Optimized proportion of mobile phase has shown good resolution for Buprofezin.

B. Method validation

The method validation was carried out as per CIPAC (Collaborative International Pesticides Analytical Council) guidelines.

Specificity: Specificity of the method was determined by injecting mobile as well as in mobile phase blank phase blank, between the peaks of active ingredients in standard, sample. Since there was no interference also peak purity was found satisfactory. Refer figure 2-4.

System suitability: System suitability is integral part of method validation. % RSD of retention times (R.T.) and peak area of five replicate injections of standard solution were less than 1.0 %.(Refer Table 2).

Table 2. System Suitability of standard solution

Parameters	Results	Limits
	Buprofezin	
% RSD of retention time	0.108	< 1.0
% RSD of peak area	0.071	< 1.0

Precision: The Precision was evaluated by repeatability. Each level of precision was investigated by five replicate injections of standard solution of Buprofezin with concentration about 1 mg/ml each and 5 different preparations of same sample. Table 3 showing acceptable % RSD values calculated by modified Horwitz equation.

Table 3. Acceptable % RSD values calculated by modified Horwitz Equation

Compound	% Analyte (m/v)	Analyte Ratio (C)	% RSD (Calc.)
Bupropion	25	0.25	1.65

Specificity : Specificity of the method was determined by injecting mobile as well as in mobile phase blank phase blank, between the peaks of active ingredients in standard, sample. Since there was no interference also peak purity was found satisfactory. Refer figure 2-4.

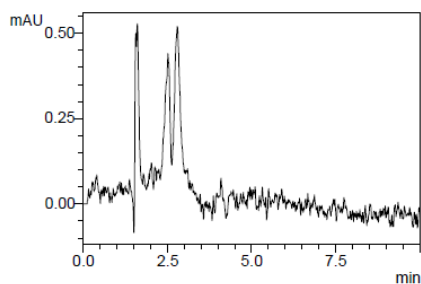


Figure 2.Chromatogram of blank

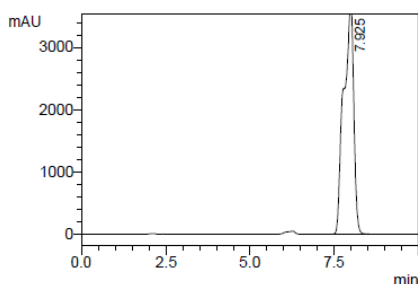


Figure 3.Chromatogram of standard preparation

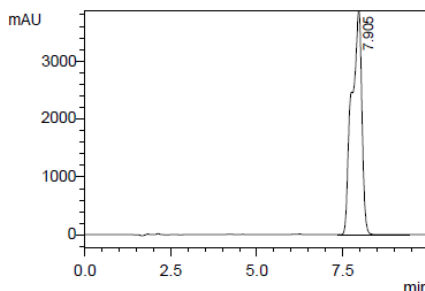


Figure 4.Chromatogram of sample preparation

Linearity : The linearity was evaluated by measuring 5 different concentration levels of standard solution of Bupropion. The linearity curve plotted concentration of standard (PPM) against mean peak areas and the correlation coefficient value was computed. The summary of the parameters were shown in Table 5 and Figure 5.

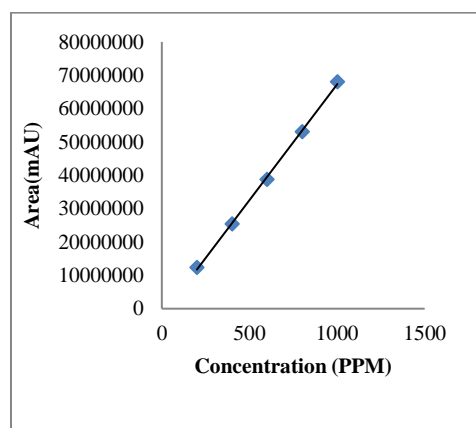


Figure 5.Graph of Linearity study

Table 5.Linearity study

Bupropion	
Linearity Range (PPM)	200- 1002
Correlation Coefficient (R ²)	0.999
Slope (m)	69409
Y-intercept (C)	2E+06

Accuracy and recovery: Accuracy (% Recovery) of analytical method was determined at four concentration levels by spiking known amount of pure actives in sample. The accuracy was calculated as % of recovery. The mean recovery results were tabulated in Table 6.

Table 6. Results of accuracy study

Components	Theoretical content of pesticide (%)	Pesticide content determined or obtained (%)	% Mean Recovery	% RSD
Buprofezin	29.36	29.32	99.87	0.681

Uncertainty in measurement (U): Uncertainty of method was measured through the data of uncertainty due to Repeatability, Calibration uncertainty of equipment or glassware, Readability of equipment, CRM purity of concentration, Linearity of calibration curve and Recovery of the analyte. The Combined Relative Uncertainty (Uc) and Expanded Uncertainty (U) were calculated [10]. Refer Table 7

Table 7. Calculated Combined and Expanded Uncertainty

Components	Mean Value (% m/v)	Combined Relative Uncertainty (Uc)	Expanded Uncertainty (U) (% m/v)
Buprofezin	24.44	0.006668	0.325878

IV. CONCLUSION

A simple, specific and reliable UFLC method has been developed for quantification of Buprofezin in their Suspension Concentrate (SC) Formulation. Method validation study showed that the method is specific, linear, accurate, and easily reproducible. This method is also useful for quantification of Buprofezin in their single or combination formulated products with

different strengths and different formulation types. This method can also be useful for analysis of environmental samples (soil, water), agricultural products for pesticide residue analysis of same actives but required additional extraction procedure and validation. Hence developed method can be adapted to regular quality control analysis of production samples and environmental samples.

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VII. Conflict of Interest

The authors declare that they have no conflict of interest.

V. REFERENCES

- [1]. Attri, M.; Qurat-ul-ain-aga, L. S.; Sharma, J.; Nesar, N. A.; Sandhu, R. S. B. R. Weed management in vegetables and flowers crops in India. 2022
- [2]. Ortelli, D.; Edder, P.; Corvi, C. Multiresidue analysis of 74 pesticides in fruits and vegetables by liquid chromatography–electrospray–tandem mass spectrometry. *Anal. Chim. Acta.* 2004, 520(1-2), 33-45.
- [3]. Correia, M.; Delerue-Matos, C.; Alves, A. Multi-residue methodology for pesticide screening in wines. *J. Chromatogr. A.* 2000, 889(1-2), 59-67.
- [4]. Egea Gonzalez, F. J.; Mena Granero, A.; Glass, C. R.; Garrido Frenich, A.; Martinez Vidal, J. L. Screening method for pesticides in air by gas chromatography/tandem mass spectrometry.

- Rapid Commun. Mass Spectrom. 2004, 18(5), 537-543.
- [5]. Armenta, S.; Quintás, G.; Moros, J.; Garrigues, S.; de la Guardia, M. Fourier transform infrared spectrometric strategies for the determination of Buprofezin in pesticide formulations. *Anal. Chim. Acta.* 2002, 468(1), 81-90.
- [6]. Buprofezin. pubchem.ncbi.nlm.nih.gov. Retrieved 26 December 2021.
- [7]. Liu, T. X.; Chen, T. Y. Effects of the chitin synthesis inhibitor buprofezin on survival and development of immatures of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). *J. Econ. Entomol.* 2000, 93(2), 234-239.
- [8]. Qureshi, I. Z.; Bibi, A.; Shahid, S.; Ghazanfar, M. Exposure to sub-acute doses of fipronil and buprofezin in combination or alone induces biochemical, hematological, histopathological and genotoxic damage in common carp (*Cyprinus carpio* L.). *Aquat. Toxicol.* 2016, 179, 103-114.
- [9]. Qureshi, I. Z.; Bibi, A.; Shahid, S.; Ghazanfar, M. Exposure to sub-acute doses of fipronil and buprofezin in combination or alone induces biochemical, hematological, histopathological and genotoxic damage in common carp (*Cyprinus carpio* L.). *Aquat. Toxicol.* 2016, 179, 103-114.
- [10]. National Accreditation Board for Testing and Calibration Laboratories (NABL). Guidelines for Estimation and Expression of Uncertainty in Measurement. 2016, 141. <http://www.nabl-india.org/nabl>
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