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Spectrophotometric detection and quantification limits of fipronil and neonicotinoids in acetonitrile

Mohamed Riad Fouad

Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University, Aflaton St., 21545, El-Shatby, Alexandria, Egypt.

Abstract

Spectrophotometer is a commonly used and basic analytical technique that can be used for both quantitative and qualitative examination in chemical, pesticides and other materials. In this work, it was studied that spectral-density (S-D) curve, calibration density (C-D) curve and quantification limits for fipronil and neonicotinoids insecticides in acetonitrile solvent on UV-Vis Spectrophotometer (Thermo Corporation, Nicolet, evolution 100) were maximum absorption (λ_{max}) equal 208, 256, 269 and 246 nm for fipronil, thiamethoxam, imidacloprid and acetamiprid, respectively. The slope and R2 were 0.059 and 0.9545 for fipronil, 0.044 and 0.9989 for thiamethoxam, 0.046 and 0.999 for imidacloprid and 0.249 and 0.9996 for acetamiprid, respectively. The limit of blank (LOB), limits of detection (LOD), and quantification (LOQ) were found to be 0.0614, 0.0623 and 0.906 µg mL-1 for fipronil, 0.0613, 0.062 and 0.0865 µg mL-1 for thiamethoxam and 0.0946, 0.954 and 0.123 µg mL-1 for imidacloprid and 0.0478, 0.0489 and 0.158 µg mL-1 for acetamiprid, respectively.

Keywords

S-D curve, C-D curve, quantification limits, fipronil, neonicotinoids.

Introduction

Spectrophotometric is a technique for determining how much light is absorbed by a chemical substance by measuring the intensity of light as it travels through a sample solution. Each substance absorbs light across a specific wavelength range. This measurement can also be used to determine how much of a known chemical compound is present. Spectrophotometry is a valuable tool for quantitative study in a variety of domains, including physics, chemistry, biochemistry, chemical engineering, and materials science. UV-visible spectrophotometers employ light in the ultraviolet range (185-400 nm), visible range (400-700 nm), and infrared range (700-15000 nm) of the electromagnetic radiation spectrum, while IR spectrophotometers use light in the visible range (400-700 nm) (Alghamdi et al., 2021). Spectroscopy is a commonly used and basic analytical technique that can be used for both quantitative and qualitative examination. UV is a quick medium of analysis that can provide excellent accuracy and precision, and it works according to the Beers-Lambert law. It can be used with a variety of chemicals and components. The ultraviolet and visible ranges of electromagnetic energy are absorbed by the sample component. These reactions are measured using visible spectroscopy. Electronic transitions can be found in molecules and atoms, while inter bond transitions in the UV and visible range can be seen in most solid objects. UV visible spectroscopy has several advantages, including accuracy, ease of handling and use, and sturdy operation. It is also a simple to use, cost-effective device that can be used in both qualitative and quantitative study. On the other hand, downsides of UV visible spectroscopy include the fact that absorption results can be impacted by (temperature, pH, pollutants, and impurities), that only liquid samples can be analysed, and that cuvette handling can affect the sample reading. The Beer–Lambert law connects the attenuation of light to the qualities of the medium it is travelling through. The law is extensively used in chemical analysis measurements and in physical optics to analyse attenuation (neutrons and photons). The linear relationship between concentration and absorbance is known as Beer's law. The Beer-Lambert law can be written as follows: $OD_{\text{absorbance}} = K_{\text{absorptivity coefficient}} * C_{\text{concentration}}$ (Oshina and Spigulis, 2021).

The detection limit in analytical chemistry and pesticide analysis refers to the smallest amount of a compound that can be distinguished from its absence. The standard deviation of the blank, the mean of the blank, and the slope of the calibration plot are used to calculate the detection limit. The standard deviation of the answer can be calculated using the standard deviation of regression lines' y-intercepts. Lower limit of detection, limit of blank (LOB), limits of detection (LOD), and quantification (LOQ) are words used to indicate the smallest concentration of a measure and that can be consistently measured by an analytical method. Defining the limits of an essay at low concentration is directly related to its analytical measurement range and dynamic range (Armbruster and Pry, 2008; El-Aswad et al., 2019). Changes in reagents, equipment, testing facilities, calibration procedures, and analysts affect data quality. As a result, users are looking for some indication of the quality of the findings, which method validation provides. When it comes to method validity, the most crucial metrics to look for are LOB, LOD, and LOQ (Armbruster and Pry, 2008; Saadati et al., 2013; El-Aswad et al., 2019). Used UV- absorption spectra to help identify organic compounds (pesticides) the lambda-max depends on the presence of light-absorbing groups in a molecule. The concentration of unknown chemicals is calculated using calibration curves based on observations of known concentration solutions. The precision and accuracy of the measurements are determined by the calibration curve. For a wide range of measurements on a wide variety of equipment, calibration curves are used.

Pest control agents, often known as pesticides, are among the most studied pollutants since they are essential in agriculture and can also be used in other fields as plant growth regulators, desiccants, and defoliants, among other things

(Kuster et al., 2006). The review covers a wide spectrum of reports on the techniques to determine pesticides in aqueous and other matrices, including extraction methods, spectrophotometric techniques, electrochemical techniques, fluorescence methods, biochemical assays, chromatographic and mass spectrometric techniques (Li et al., 2020). Bustamante et al. (2019) designed polymer films containing diazonium salts anchored chemically for colorimetric determination of 2-phenylphenol derivatives in water. Which was quantified with a UV-Vis spectrophotometer with a LOD of 0.12 µg mL^{-1}. Among agricultural insecticides, fipronil and neonicotinoids are the most used for several crops and fruits due to their excellent insect control efficacy (Tomazini et al, 2020). LOD from 0.002 to 0.006 mg g^{-1} and LOQ from 0.006 to 0.020 mg g^{-1} were reached for the pesticide fipronil and its intermediates fipronil desulfinyl, fipronil sulfone and fipronil sulfide, in soil by Tomazini et al, 2020. In honey samples, the LOD and LOQ for seven neonicotinoids (thiamethoxam, dinotefuran, nitenpyram, acetamiprid, clothianidin, imidacloprid, and thiacloprid) were respectively 1.5–2.5 mg kg^{-1} and 5.0–10.0 mg kg^{-1} (Jovanov et al., 2015).

Materials and Methods

Tested insecticides

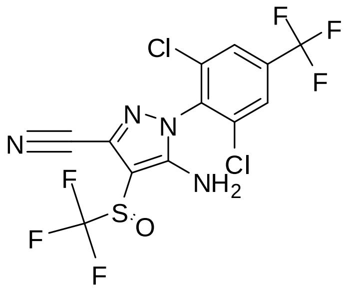
Fipronil

IUPAC name: 5-Amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4 (trifluoromethanesulfinyl)-1*H*-pyrazole-3-carbonitrile. The chemical structure: (Fig. 1) Technical 99 % a.i. Solubility (20 ºC) in water 3.78 mg/L. Production company: EGYPTCHEM, Egypt.

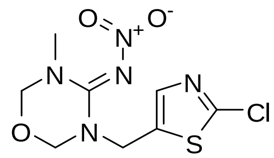
Neonicotinoids

Thiamethoxam, IUPAC name: {3-[(2-Chloro-1,3-thiazol-5-yl) methyl]-5-methyl-1,3,5-oxadiazinan-4-ylidene} nitramide. Technical 98.5 % a.i. Solubility in water 4100 mg/L. Production company: EGYPTCHEM, Egypt. **Imidacloprid**, IUPAC name: *N*-{1-[(6-Chloro-3-pyridyl) methyl]-4,5-

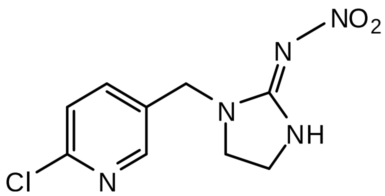
dihydroimidazol-2-yl} nitramide. Technical 95 % a.i. Solubility in water 510 mg/L at 20 ºC. Production company: AGROCHEM, Egypt. **Acetamiprid,** IUPAC name*:* *N*-[(6-chloro-3-pyridyl) methyl]-*N'*-cyano-*N*-methyl-acetamidine. Technical 97 % a.i. Solubility in water 2950 mg/L. Production company: KAFR ELZAYAT, Egypt. The chemical structure of neonicotinoids is depicted in Figure 1.



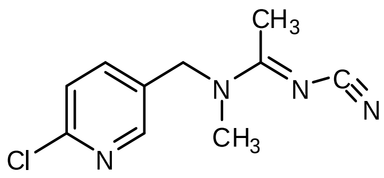
Fipronil



Thiamethoxam



Imidacloprid



Acetamiprid

Fig. 1. Chemical structures of tested insecticides.

Determination of tested insecticides

Each insecticide's standard solution (0.1–25 µg mL^{-1}) was made by dilution of the stock solution 500 µg mL^{-1} dissolved in acetonitrile solvent. The optimum wavelength (λ_{max}) was generated using a UV-Vis Spectrophotometer with a scanning range of (200–400 nm). The standard calibration curve (C-D curve) was generated by graphing the concentrations against their matching absorbance at the maximum concentration (max). Triplicate samples, including controls and blanks, were used for quality assurance.

Insecticides detection and quantification limits

A spectral density curve was used to test the specificity of the spectrophotometric approach for pesticide determination (S-D curve). The limit of blank (LOB), limit of detection (LOD), and limit of quantitation (LOQ) are essential terms that describe the smallest pesticide concentration that can be consistently detected under experimental conditions. They were determined using the equations below (El-Aswad et al., 2019).

$$LOB = Mean_{blank} + 1.645\ SD_{blank} \quad (1)$$

$$LOD = LOB + 1.645\ (SD_{low\ concentration\ sample}) \quad (2)$$

$$LOQ = (SD/S)*10,\ LOQ \geq LOD \quad (3)$$

At λ_{max}, ten pesticide-free blank samples were measured, and their averages and standard deviations (SD blank) were determined. To compute SD low concentration sample, ten samples of the lowest concentration that reliably detected above blank absorbance were measured. The absorbance of a pesticide concentration slightly greater than LOD was measured and found to be consistent and dependable. The slope (K) of the calibration graph (S) and the standard deviation of the response (SD) of the curve were used to compute the LOQ.

Results and Discussion

A characteristic value, indicated as λ_{max}, is the wavelength of maximum absorption. The absorption spectrum for fipronil insecticide in acetonitrile showed maximum absorption (λ_{max}) at 208 nm (Fig. 2-A). The absorption spectrum for fipronil in distilled water with diode array spectrophotometer was λ_{max} 208 nm by Ngim and Crosby, (2001). The standard curve is a method for measuring the concentration of a drug in an unknown sample by comparing it to a series of known concentration standard samples. The calibration curve of fipronil showed in Fig. 2-B where the correlation coefficient is 0.9547, K = 0.059 µg mL^{-1} and the intercept from straight line was 0.14 µg mL^{-1}. Was the working scanning and calibration by spectrophotometer of insecticide according to Beer Lambert Law (Adeeyinwo et al., 2013). Table 1 show that the LOB, LOD, and LOQ for fipronil are 0.0614, 0.623, and 0.0906 µg mL^{-1}, respectively.

The findings of the study matched those of Lacroix et al. (2010) and He et al. (2021) in the literature.

Thiamethoxam, a member of the thia-nicotinyl subclass, is the first of the second-generation neonicotinoids. It's sold as Actara® for soil and foliar applications (Mir et al., 2013). The absorption band of thiamethoxam is distinct, with a maximum at 256 nm. Consequently, the 256 nm absorption was used in the study for calibration density (Fig. 3A & B). Mir et al (2013) monitored the photocatalyzed degradation of thiamethoxam in water by measuring the change in absorbance at its maximum wavelength of 251 nm. Up to a concentration range of 0.5–25 μg mL^{-1} in acetonitrile, the calibration graph was linear (R^2 = 0.999). The slope and intercept were found to be 0.0437 and 0.0056 μg mL^{-1} for thiamethoxam. In Table 1, detection and quantification limits for the pesticide thiamethoxam were derived as the lowest point on standard curves with relative standard deviations. The quantification limits of thiamethoxam 0.086 for LOQ, 0.062 for LOD and 0.062 for LOB by Uv - spectrophotometer. Abd-Alrahman determined the LOD and LOQ of thiamethoxam in vegetables using HPLC to be 0.02 and 0.06 mg kg1, respectively (Abd-Alrahman, 2014).

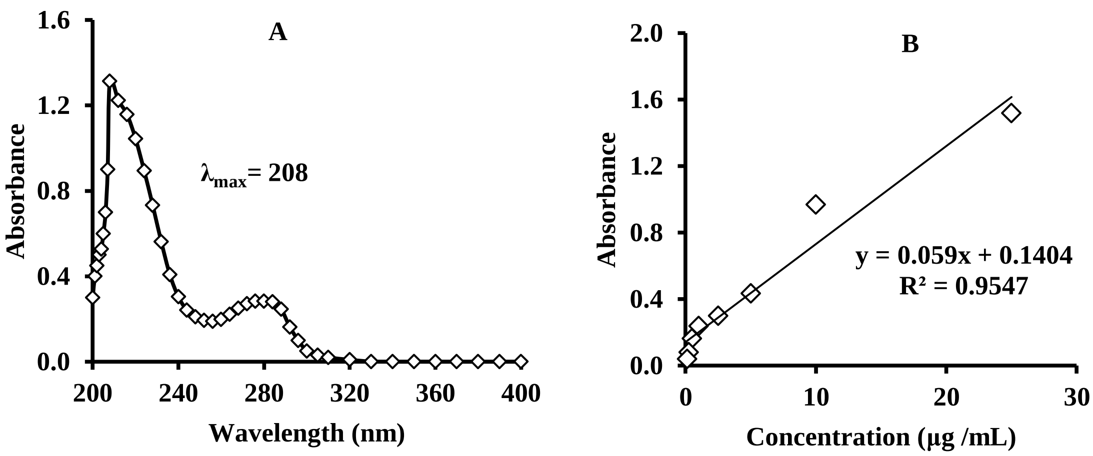


Fig. 2. Fipronil's spectral-density (S-D) curve (A). The maximal wavelength is 208 nm, and the calibration density (C-D) curve of fipronil (B) at 208 nm is readily visible using the UV-spectrophotometric method.

Table 1 Fipronil and neonicotinoids detection and quantification limits

Insecticide	Fipronil	Thiamethoxam	Imidacloprid	Acetamiprid
Mean $_{\textbf{blank}}$	0.059	0.060	0.094	0.046
SD $_{\textbf{blank}}$	0.0008165	0.0008165	0.000488	0.0007868
SD $_{\textbf{low concentration sample}}$	0.0005345	0.000378	0.0005345	0.0006901
LOB	0.061343	0.061243	0.094517	0.047723
LOD	0.062222	0.061965	0.095396	0.048858
LOQ	0.090597	0.086491	0.122316	0.15791

Imidacloprid belongs to the most efficient class of insecticides (neonicotinoids) nowadays which account for about (17 %) of the total insecticide market (Guzsvány et al., 2009). Figure 4 depicts the S-D and C-D curves for imidacloprid. The maxima wavelength is 269 nm, the slope (K) was 0.0458 μg mL^{-1} and correlation coefficients were 0.999 for imidacloprid by spectrophotometer (Fig. 4-A&B). The entire optical absorption spectrum of imidacloprid in water was recorded, and it was discovered that imidacloprid has a maximum absorption wavelength of about 270 nm (Daneshvar et al., 2007). The LOB, LOD and LOQ were in acetonitrile found to be 0.0946, 0.954 and 0.123 μg mL^{-1} for imidacloprid in Table (1). Bonmatin et al. reported that the limits of detection (LOD) and quantification (LOQ) for imidacloprid in maize crops were 0.1 and 1.0 g kg^{-1}, respectively (Bonmatin et al., 2005).

Acetamiprid is a systemic neonicotinoid insecticide used to suppress sucking insects in citrus, leafy vegetables, pome fruits, cotton, grapes, and other crops and ornamental plants. Figure (5) shows the maximum wavelength and calibration density curve for acetamiprid. For acetamiprid, the maximum wavelength is 246 nm, the slope is 0.2488 μg mL^{-1}, and the correlation coefficients are 0.9996 (Fig. 5-A&B). Using a Shimadzu UV–Vis Spectrophotometer and HPLC, the degradation of acetamiprid was monitored by detecting the change in absorption intensity at its maximum (245 nm) (Khan et al., 2010). The LOB, LOD and LOQ were found to 0.0478, 0.0489 and 0.158 μg mL^{-1} for acetamiprid, respectively (Table 1). The LOD and LOQ found were 0.83 - 1.7 μg L^{-1} for acetamiprid in black currant juice samples by Tursen et al. (2021).

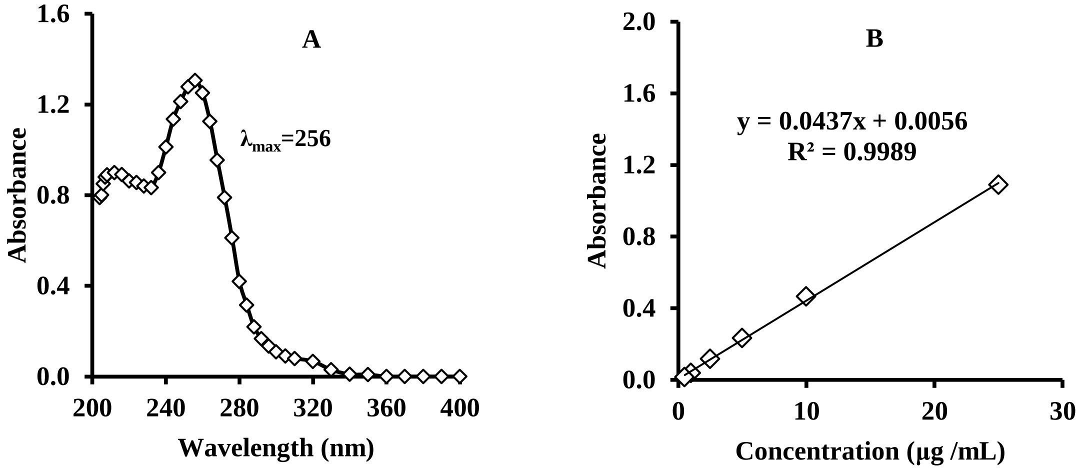


Fig. 3. Thiamethoxam spectral-density (S-D) curve (A). The maximum wavelength is 256 nm, and the calibration density (C-D) curve of thiamethoxam (B) at 256 nm is readily visible using the UV-spectrophotometric method.

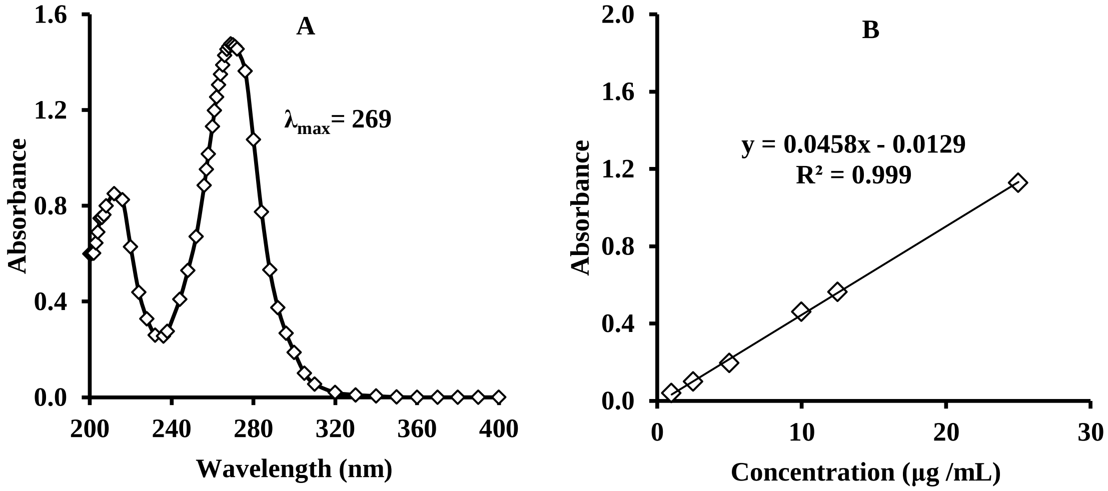


Fig. 4. Imidacloprid's spectral-density (S-D) curve (A). The maximal wavelength is 269 nm, and the calibration density (C-D) curve of imidacloprid (B) at 269 nm is clearly visible using the UV-spectrophotometric method.

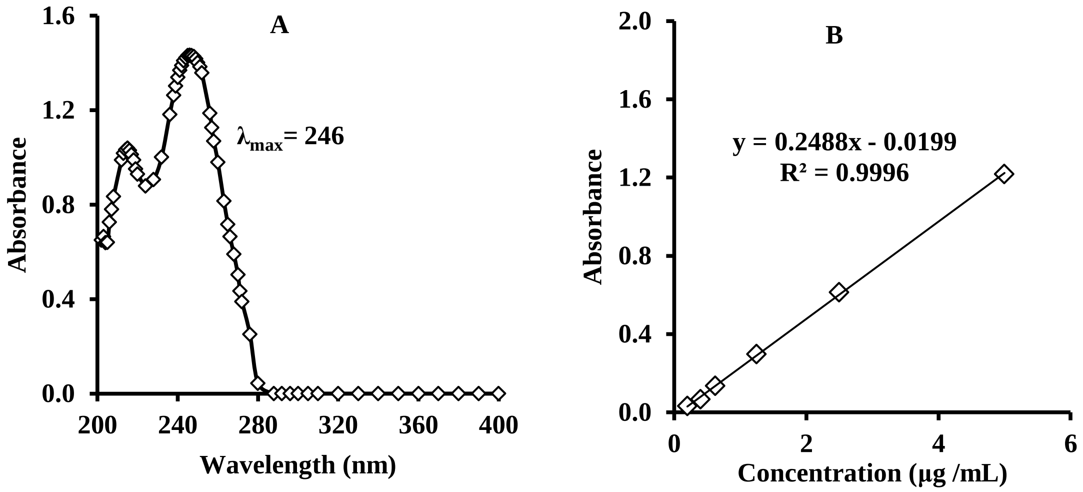


Fig. 5. Acetamiprid's spectral-density (S-D) curve (A). The maximal wavelength is 246 nm, and the calibration density (C-D) curve of acetamiprid (B) at 246 nm is clearly visible using the UV-spectrophotometric method.

Conclusion

It is important to know the quantification limits for insecticides, especially fipronil, thiamethoxam, imidacloprid and acetamiprid, the most commonly used in the agricultural field. Scanning and fixed these insecticides found that λmax equals 208, 256, 269 and 246 nm for fipronil, thiamethoxam, imidacloprid and acetamiprid, respectively. The K was 0.059 for fipronil, 0.044 for thiamethoxam, 0.046 for imidacloprid and 0.249 for acetamiprid. The quantification (LOQ) was found to be 0.906 µg mL^{-1} for fipronil, 0.0865 µg mL^{-1} for thiamethoxam, 0.123 µg mL^{-1} for imidacloprid and 0.158 µg mL^{-1} for acetamiprid.

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