2015 International Nuclear Atlantic Conference - INAC 2015
São Paulo,SP, Brazil, October 4-9, 2015
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-06-9**

ANALYSIS OF NEONICOTINOIDS BY GAS CHROMATOGRAPHY COUPLED TO NUCLIDE ^{63}Ni – ELECTRON CAPTURE DETECTOR – GC/ECD

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ABSTRACT

Recently, several reports have been published discussing reduction in bee population which polymerizes cultures around the world this phenomenon is known as Colony Collapse Disorder (CCD). The phenomenon describes the lack of worker honeybees in the colony despite having pups and food. The causes of this problem are unknown but there are studies that claim that reduction of population of bees is linked to poisoning through insecticides specifically neonicotinoids. Among this type of pesticide are imidacloprid ($C_9H_{10}ClN_5O_2$), clothianidin ($C_6H_8ClN_5O_2S$) and thiamethoxam ($C_8H_{10}ClN_5O_3S$). This paper presents the analysis of neonicotinoids – clothianidin, imidacloprid and thiamethoxam - by the technique of gas chromatography coupled to nuclide ^{63}Ni electron capture detector (GC/ECD). The electron capture detector (ECD) is a gas chromatography detector that has been used for the detection of organic halogens, nitriles, nitrates and organometallic compounds. The ECD detector ionizes the analytes by the beta particles from the nuclide sources ^{63}Ni within carrier gas N_2. The electrons produced in this process are collected and create a current that are amplified and generates a chromatographic peak. Methodology and details of the analysis are present in this work.

1. INTRODUCTION

Since 2012 are being published several articles discussing the reduction in the population of bees that polymerize cultures around the world, this phenomenon is known as Colony Collapse Disorder (CCD) or Mad Bee Disease. The Mad Bee Disease began to manifest in 1994 in France, when his bee population began to show disorientation, agitated behavior and difficulty to find the hive again [1]. The Colony Collapse Disorder is the phenomenon that describes the absence of adult honey bees in the colony although there pups and food, or where the amount of immature bees (brood) is greater than the amount of workers' bees [2].The causes of this problem are unknown but there are studies that claim that reduction of population of bees is linked to poisoning through insecticides, specifically neonicotinoids. For that reason in 24 May 2013 the European Union amended the Commission Implementing Regulation No 540/2011 prohibiting the use and marketing of seeds treated with the active substances clothianidin, imidacloprid and thiamethoxam.

The importance of this prohibition is reflected in the fact that bees are the main pollinators of nature. Pollination is the transfer of pollen (male gamete) to the stigma (female gamete) for the same flower or to another flower of the same type in order to begin the formation of fruits and seeds of a plant [3]. An estimated 75% of crops and 80% of the plant species that provided flowers are dependent of animal pollination, and 73% of agricultural crops in the world are pollinated by bees [4].

The neonicotinoid class of insecticides is based on the nicotine molecule. These classes of insecticides are now representing the largest selling class of insecticide and seed treatments on the global market. They can be conveniently used as seed or in furrow treatment to protect crops from sucking and chewing insects [5].

Neonicotinoids insecticides are successful because of the low toxicity to mammals but they are extremely toxic to most insect pests. All neonicotinoids bind to the post-synaptic nicotinic acetylcholine receptors (nAChRs) in the invertebrate central nervous system, competing with the natural neurotransmitter acetylcholine (ACh). Toxicity studies with arthropods suggest that the binding to these receptors is long-lasting, and lethal effects are typically delayed [5]. The selected compounds for these analyzes are: clothianidin ($C_6H_8ClN_5O_2S$), imidacloprid ($C_9H_{10}ClN_5O_2$) and thiamethoxam ($C_8H_{10}ClN_5O_3S$).

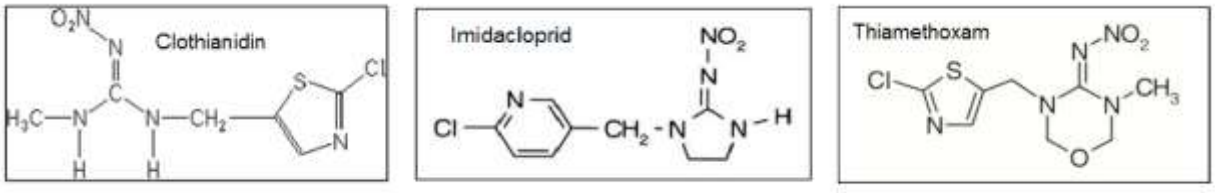

Figure 1: Molecular structure of clothianidin, imidacloprid and thiamethoxam.

A suitable method for the separation and identification of these compounds is gas chromatography coupled to electron capture detector (ECD). Gas chromatography is based on the distribution of substances in a sample from a stationary phase (solid) and a mobile phase (gas) [6]. The main parts of a gas chromatograph are: injector, column and detector.

The sample goes through an injection system then is introduced in a column containing the stationary phase. The use of temperatures in the sample injection and column makes it possible to spray these substances that, according to their properties and the stationary phase are retained for a time and emerge out of the column at different times [6] well known as Retention Time. Immediately after sample pass through the detector the analytes identifies by generating a signal. The set of these signals is represented by a chromatogram.

The electron capture detector (ECD) is a composite detection device that uses electrons generated by a radioactive source, generally nickel-63. When the carrier gas (N_2) passes through the detector, the carrier gas is ionized by particles beta (β) that are emitted by the source of Ni 63 attached to the detector. The electrons produced in this ionization are collected at an anode and generate a current which is amplified and results in a baseline. The analytes that can capture electrons are eluted and this decreases the base current line, generating a signal proportional to its concentration [6].

This detector presents a good selectivity halogenated molecules (molecules with fluorine, chlorine, bromine or iodine), conjugated carbonyls, nitro compounds and organometallics. The ECD is virtually insensitive to hydrocarbons, alcohols and ketones. The ECD detector scheme is shown in Figure 2 [6].

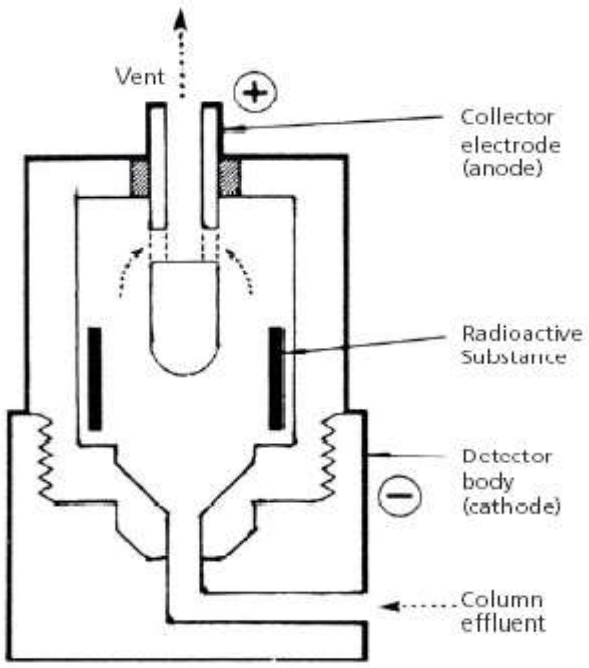

Figure 2: Electron capture detector structure (ECD)[7].

The objective of this work is to develop a methodology for neonicotinoids - thiamethoxam, clothianidin and imidacloprid - using gas chromatography coupled to electron capture detector (GC/ECD).

There is no legislation for these compounds in Brazil, however the EPA (Environmental Protection Agency) of the United States establishes that these compounds should be in 10 ppb for chronic exposures.

2. INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

To make the solution used in these analyzes were used reference materials of imidacloprid, clothianidin and thiamethoxam from the company Sigma Aldrich with 99% purity. From these patterns was made a solution by mixing these compounds in a concentration of 10 mg / L. These solutions were diluted with acetonitrile for chromatographic analysis on a GC / ECD Shimadzu model GC17A. Initially, three microliters were injected into the gas chromatograph, the injection method used was split 1:10, the column used was DB5 (5% siloxane), the detector temperature is 280°C and the pressure in the column was 100 kPa through the chromatographic run. The total analysis time is 21.33 minutes and the temperature curve will be described in the table below.

Table 1: The temperature curve of GC 17 A.

Rate (°C/min)	Temperature(°C)	Isothermal (min)
-	50	2
10	60	4
10	100	1
30	280	2

3. RESULTS AND DISCUSSION

3.1. Retention time of Neonicotinoids

The first analyzes were performed to determine the retention time of each compound, this analysis used an individual concentration of 50 mg / L of the pesticides and a mixture of the three neonicotinoids with a concentration of 10 mg / L . A higher concentration of the individual analytes was used in order to compare the highest intensity peaks with the peaks identified in the mixture. The retention times obtained were: 8.3 min for imidacloprid, 15.3 min for clothianidin for and 22.1 min for thiamethoxam. Figure 3 depict these analyses.

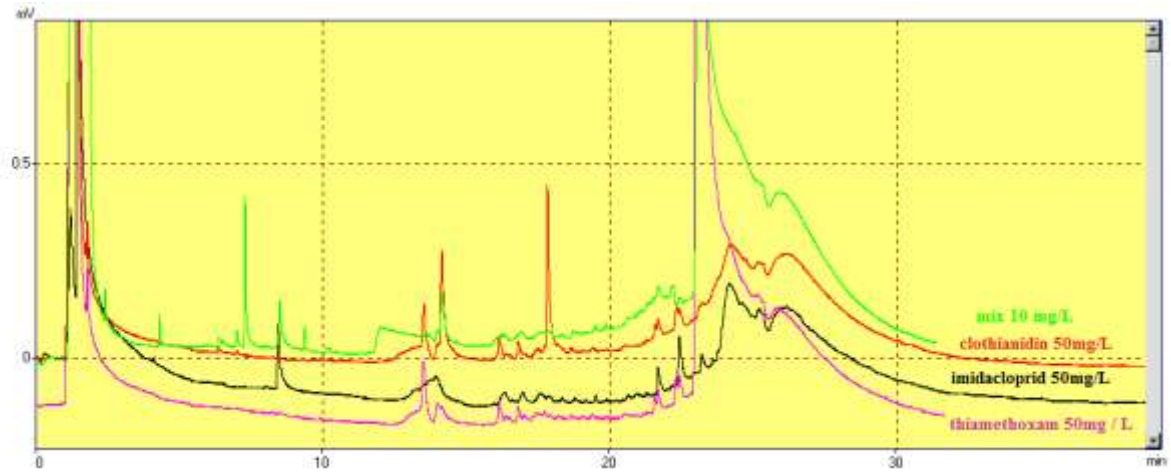

Figure 3: Comparison of the chromatograms for each pesticide by GC / ECD

3.2. Sample Injection Volume Tests

Other tests were performed to see if there was any relation between increased injection volume and the intensity of the analyzed peaks. The analyses were made with injections of 1, 2, 3 and 4 microliters of a mix solution of 10 mg / L. These results and comparison are on Table 2.

Table 2: Comparison between the sample injection volume and the chromatographic peak area

Injection Volume (µL)	Imidacloprid Peak Area	Clothianidin Peak Area	Thiamethoxam Peak Area
1	495	2327	14701
2	584	1408	24357
3	932	1438	26108
4	556	2914	11466

Observing the table it can be concluded that the volume of 3 microliters is the ideal volume for these analyzes, and the increase in the injection volume cannot guarantee an increase in resolution or chromatographic peak area.

3.3. Temperature Tests

A mix standard with 10 mg/L was used to temperature tests. This test consists in varying the temperature stabilization of the temperature ramp from 100 and 120° C to verify if there is any change in the identification or the peak intensity obtained in these analyzes. The obtained chromatograms can be seen in Figure 4.

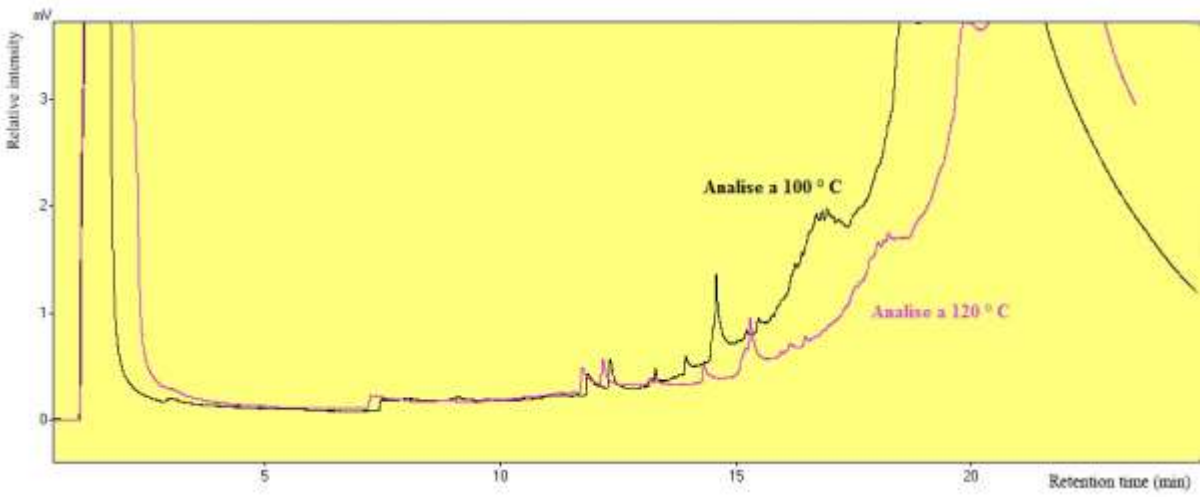

Figure 4: Comparison of chromatograms with different temperatures.

From these chromatograms is possible to determine that stabilize the temperature of 100 ° C is the most suitable for the temperature ramp of this method due to the fact that the analysis at 100 ° C has a higher peak intensity and a lower retention time than the analysis performed at 120 ° C. It can also be compared in Table 3 and Table 4.

Table 3: Comparison between the analysis in different temperatures and the retention time of the neonicotinóides

Stabilized temperature on the rate (° C)	Imidacloprid Retention Time (min)	Clothianidin Retention Time (min)	Thiamethoxam Retention Time (min)
100	12.306	14.561	18.525
120	12.150	15.283	19.840

Table 4: Comparison between the analysis in different temperatures and the chromatographic peak area

Stabilized temperature on the rate (° C)	Imidacloprid Peak Area	Clothianidin Peak Area	Thiamethoxam Peak Area
100	1879	5803	17767
120	1485	5622	12936

3.4. Lowest concentration for neonicotinoids by GC/ECD

At last, tests were performed to determine the lower detection limit for the three neonicotinoids studied by the method of GC / ECD. The lowest concentration analyzed was 0.7 mg/L and the tested concentrations were between 0.7 mg/L and 500 mg/L. The chromatogram of the 0.7mg/L for the mix can be seen on figure 5.

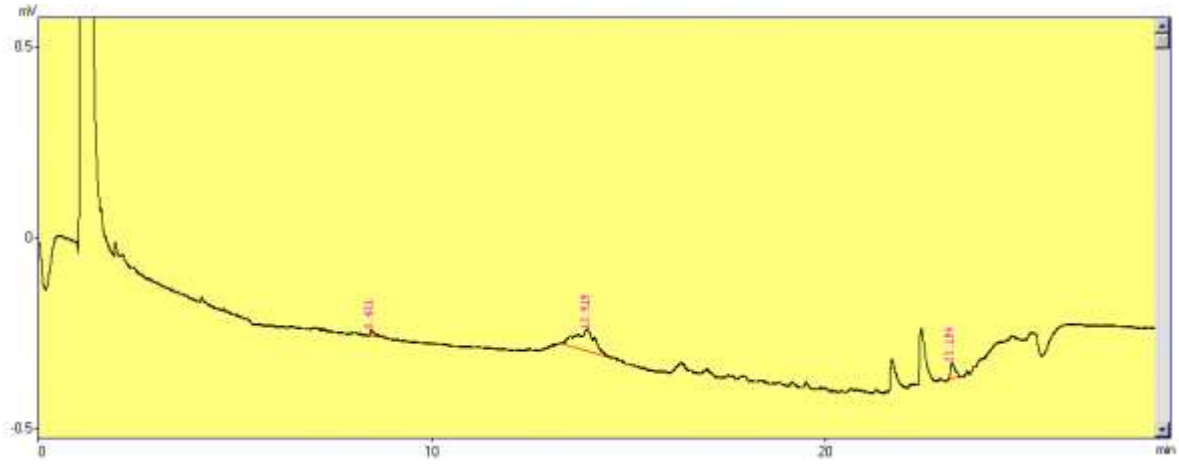

Figure 5: Chromatogram with neonicotinóides mix of 0.7 mg/L

From this chromatogram it is possible to verify that the GC / ECD cannot analyze amounts of pesticides lower than 0.7 mg / L. This makes it impractical to use this method in water analyzes because this type of analysis needs to be validated with concentrations in the order of µg / L.

4. CONCLUSIONS

From these analyzes can be concluded that the GC / ECD can identify neonicotinoids with a good efficiency and resolution but a higher sensitivity would be required to perform the amounts required by the Environmental Protection Agency (EPA) in the environment.

Because the objective of this study is a method validation for water analysis with neonicotinoids, it would be necessary to use another analytical method that should be more sensitive for these compounds, for example, the liquid chromatography coupled with mass spectrometry tandem (LC- MS/MS) or the liquid chromatography coupled with an ultraviolet detector (LC-UV).

ACKNOWLEDGMENTS

We would like to thank Improvement Coordination Higher Education Personnel (CAPES) for the master scholarship.

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