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HALOGENATED PESTICIDE ANALYSIS IN ORANGE JUICE BY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTOR (GC-ECD) WITH ^{63}Ni NUCLIDE

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ABSTRACT

Brazil has been gain space in the market of orange juice in the last years. For the exportation of this product to keep growing, its quality of this product must be ensured by puting in force more strictive legislations and custom barriers, in order to improve the well-being and health of the population. In this work were analyzed four orange juices brands produced in the State of São Paulo. It was quantified the acaricide known as Dicofol (2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol) widely used to combat the *Citrusleprosis* virus, transmitted by mites to the citrus culture. This pesticide was chosen due to its importance in the production of orange in large scale and their indiscriminate use may pose risks to humans and of environment. The analytical technique applied was gas chromatography coupled with electron capture detector (GC-ECD) using the ^{63}Ni nuclide. This beta (β) radiation source ionizes the carrier gas (N_2), generanting an electron current that forms the baseline. The analites pass through the detector and capture electrons, generating the analitycal signal that is proportional to the concentration of analite. The sample preparation was done by QuEChERS. The limits of detection (LOD) and quantification (LOQ) found were 0.005 and 0.025 mg kg^{-1} respectively. The applied methodology was efficient and presented excellent analytical sensitivity for the pesticide Dicofol, being that of four samples analyzed, only in one was found concentration of 0.03 mg kg^{-1}, above the LOQ, however below the Maximum Residue Limit (MRL) for fruits (0.1 mg kg^{-1}) established by *Codex alimentarius.*

Key-words: GC/ECD, Dicofol, QuEChERS, Orange juice.

1. INTRODUCTION

Brazil is the world`s biggest orange juice exporter, being responsible for 60 % of the global production, among which 85 % is consumed only by United States, European Union and Canada. The concentraded orange juice is the main industrial product from the fruit. Other products are essencial oils and aromatic liquids. The citrus bagasse is sold as an industrial by-product, with high calorific power and significant economic value, used in the production of animal feed for rumminants, especially milky cows. (OECD/FAO, 2015), (Lohbauer, et al. 2011).

With the increasing Brazilian export potencial, government and private sector gives great importance to multiresidue analysis of pesticides. To surpass the customs barriers and fulfill the strict export legislation, it is necessary improvements in analytical methodologies, reduction of costs and quick results (ANVISA, 2006), (Pizzutti, 2006).

Duo to the importance of analytical development for the pesticide multiresidue analysis, this present study aimed to quantify of the Dicofol pesticide in orange juice produced in the State of São Paulo, using gas chromatography with electron capture detector (GC-ECD) with ^{63}Ni nuclide, considering the sensitivity of this technique.

1.1 Dicofol characteristics and legislation

Dicofol, IUPAC chemical name 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol (Pubchem, 2016) is a substance with acaricide characteristics, belonging to the organochlorine chemical family (Fig. 1).

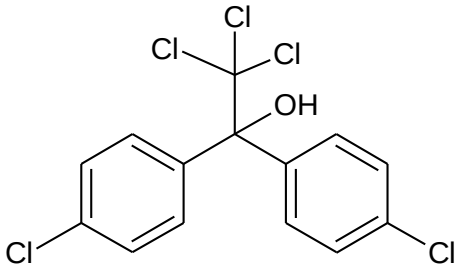

Figure 1: Chemical structure of Dicofol (2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol). Source: Pubchem, 2016; Autor

This pesticide is used to combat plagues such as the false rust mitte (*Phyllocoptruta Oleivora)* (Fig. 2), one of the main plagues for fruits from the citrus family. (Azevedo,et al 2006).

Figure 2: Orange tree with the false rust plague, compromising the entire fruit. Superimposed, the image of the false rust mitte. (Azevedo, et al 2006).

However public health studies indicate that exposure to organochlorine pesticides can cause hepatic and renal damage, peripheral neuropathies and cancer (Muller et al, 2012).

1.2 .Sample preparation

The sample preparation was applied by QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). This method was developed by Anastassiades et. al in 2003, being widely disclosed since then, because it can be applied for high complexity samples such as food. Prestes et. al in 2011, compared several modern sample extraction techniques and concluded that QuEChERS represents the state fo the art in the pesticide multiresidue determination for foodstuff. This preparation technique makes samples ready for any chromatographic analysis (Prestes et. al, 2011).

1.3. GC-ECD analysis

The electron conductivity detector was invented in 1958 by the English Chemist, doctor and scientist James E. Lovelock. His motivation was development a detector more sensible for gas chromatograph using the ionization of particles. At the beginning, the detector consisted in a small cilindric chamber, with a central brass tube that worked as anode. This chamber was kept aligned with a silver sheet coated with Strontium nuclide (^{90}Sr), that worked as a beta radiation source (β) and also as a cathode. For safety and costs reasons Lovelock used Nitrogen as carrier gas. During the experiments, he discovered that substances such as carbon chloride (CCl_4) among other organochlorine compounds capture electrons when passing through the detector, consuming its signal. Based on this observation, Lovelock concluded that the new device was a thousand times more sensible than the Argon detector, popular at the time. The ECD detector has passed through several improvements, with the ^{63}Ni ionizing radiation source, being the most expressive one (Lovelock,1958); (Sella, 2015); (Rafferty, 2015).

This analytical technique is very sensible to halogenated compounds such as organochlorine and organophosphorous compounds due to it's afinity for electrons (Miao et. al. 2013). The compounds of interest are separated in the gas chromatograph using a temperature ramp and by means of physicochemical interactions between the analytes and the stationary phase of the chromatographic column. The ECD detector has the ^{63}Ni source that emmits β ionizing particles (Fig. 3). The carrier gas (N_2) coming from the chromatographic system reaches the detector and ionizes when it pass through the beta radiation beam releasing electrons. This electrons are collected in an anode that generates an electric current. This current is amplified by an electrometer and the baseline is formed. When an halogenated susbstance pass through the electron beam it captures the electrons, decreasing the current that forms the baseline. This disturbance in the baseline is proportional to the concentration of the analytes. ECD is less sensitive to hidrocarbons, alcohols and ketones, because these compounds have little or no afinity for electrons, what makes this detector more selective and suitable for the analysis of organochlorine pesticides such as Dicofol (Collins et. al, 2006).

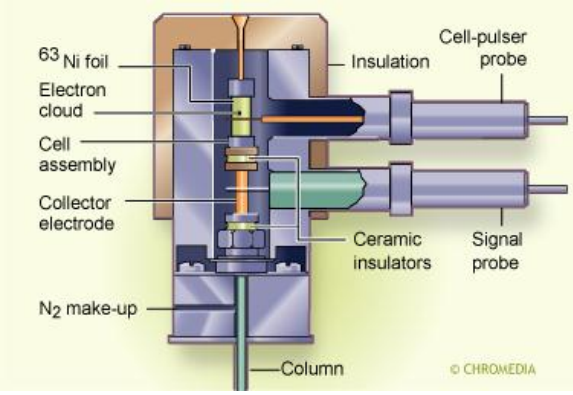

Figure 3: The electron capture detector – ECD. (Source: Chromedia Analytical Sciences, 2017).

Alves et. al in 2012, compared gas chromatography-mass spectrometry methods (GC-MS with selective ion monitoring) with the GC-ECD technique for organochlorine and organophosphorus pesticide multiresidue analysis in Brazilian citric essencial oils. Among the analyzed pesticides is Dicofol. The conclusion about this comparison was GC-ECD had more sensitivity to these analytes. In his research, it was shown that the limits of detection (LOD) and quantification (LOQ) for GC-ECD were smaller than those for GC-MS, with a value less than the MRL for Dicofol.

Miao et. al in 2013, developed the multiresidue analysis for pesticides in lotus flower using QuEChERS as the sample preparation technique, and analyzed by GC-ECD with confirmation by GC-MS. In his work, he determine LOD and LOQ of 0.002 and 0.005 mg kg^{-1}, respectively, for Dicofol, besides reporting the method as linear, selective, reproducible, stable and good recovery for multiresidue analysis.

The ANVISA (National Agency for Health Surveillance) is responsible to regulates the use and control of pesticides in Brazil (ANVISA, 2012**)** and the FAO-ONU (Food and Agriculture Organization of the United Nations) is linked with *Codex Alimentarius*, which is international organization that regulates the standards and directives for foodstuff (*Codex Alimentarius*, 2017). The Maximum Residue Limit (MRL) is the maximum amount of agrotoxic residue allowed for specific types of food, after the use of permited pesticides and in specific stages of production and this value can be expressed in mg kg^{-1} (ANVISA, 2012**).** The ANVISA established a MRL for Dicofol in citrus culture as 5 mg kg^{-1} (MAPA, 2017) and *Codex Alimentarius* established that 0.1 mg kg^{-1} for fruits (*Codex Alimentarius*, 2017). As there is no specific limit for orange juice, the reference value adopted in this work will be the more stringent one, which is the Codex limit. The choice of this limit of reference aims to observe the sensitivity of the GC-ECD technique.

2. EXPERIMENT

2.1 Materials and Reagents

The following reagents and materials were applied: acetonitrile, HPLC grade ≥ 99,9%; from Sigma Aldrich; Acetone HPLC grade ≥ 99,9%, from Sigma Aldrich; primary secondary amine (PSA) HPLC grade, from Agilent Technologies; Magnesium sulfate anidrous, reagent

grade ≥ 99,5% from Sigma Aldrich; Sodium Chloride from Carlo Erba; PTFE syringe filter 0,45µm from Allcrom; 2 mL screw cap vials; 50 µL, 250 µL, 500 µL microsyringes from Hamilton; Dicofol 100 µg/mL in ethyl acetate from Dr. Ehrenstorfer, ultra pure nitrogen (99,9999%) from Linde; ultrapure water with resistivity ≥ 18Ωm (this water was obtained through a purification equipment of laboratory – System MilliQ Advantage), 50 mL e 15 mL Falcon tubes.

2.2 Equipment

For sample preparation it was used: analytical balance, Mettler Toledo AG 245 model; Centrifuge, FANEM 206 model; Agitator, Phoenix Luferco AP56 model and purification equipment of ultrapure water, System MilliQ Advantage A-10 model.

It was used a Shimadzu GC-ECD, GC-2010 Plus model with ^{63}Ní radionuclide, autosampler AOC-20i, capillary column Equity TM-1701 30 m x 0,53 mm x 1,0 µm from Supelco.

The chromatographic conditions were:
1 µL injection volume; injection mode - split ratio 5; injector temperature: 220 ºC; carrier gas (N_2): 5.72 mL min^{-1}; Oven program: 120 ºC for 1 min, 20 ºC min^{-1}. to 170 ºC, kept for 1 minute / 2 ºC min^{-1}. to 224 ºC, kept for 4 minutes / 35 ºC min^{-1}. to 250 ºC, kept for 1 minute. The injections were made through the automatic injector. The samples and standards were injected three times into the equipment.

2.3 Samples

All samples were bought in local shops at Butantan neighbourhood, São Paulo-SP. It was choosen only juices produced in the State of São Paulo. The sample were identified as shown in Table 1.

Table 1. Identification of orange juice samples

Sample name *	Type of juice
Sample N	Fresh juice, extracted direct from the fruit.
Sample O	100% organic orange juice
Sample Q	Concentrated orange nectar with 50% of juice
Sample C	Concentrated orange nectar with 40% of juice

***The samples were renamed to avoid the disclosure of brands.**

2.4 Calibration curve preparation

A stock solution of Dicofol at concentration of 1.0 mg kg^{-1} was prepared in a 25 mL amber volumetric flask, by diluting 0.25 mL of the standard solution (Dicofol 100 µg mL^{-1} in ethyl acetate), the volume was completed with acetone HPLC grade. The standard solution was then stored in a refrigerator at 5 ºC.

The calibration curve was prepared from the stock solution at the following concentrations: 0.005, 0.01, 0.025, 0.05, 0.1, 0.2 and 0.3 mg kg^{-1}, respectively, in acetonitrile HPLC grade.

2.5 Sample preparation

The samples were prepared according to the flowchart in Fig. 4. For the spiked samples it was added 0.1 mg kg^{-1} of the standard Dicofol before the first extraction step.

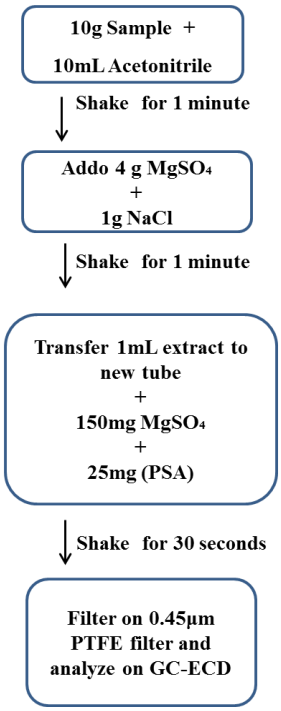

Figure 4: Flowchart for the QuEChERS extration (Source: author, adapted from Anastassiades, 2003)

2.6 Limit of detection and limit of quantification

For the sensitivity evaluation of the instrument, the LOD and LOQ were determined. Were considered a LOD and LOQ of 0.005 and 0.025 mg kg^{-1}, respectively, for Dicofol.

3. RESULTS AND DISCUSSION

The lower concentration detected was 0.005 mg kg^{-1}, according to the chromatogram in Fig. 5.

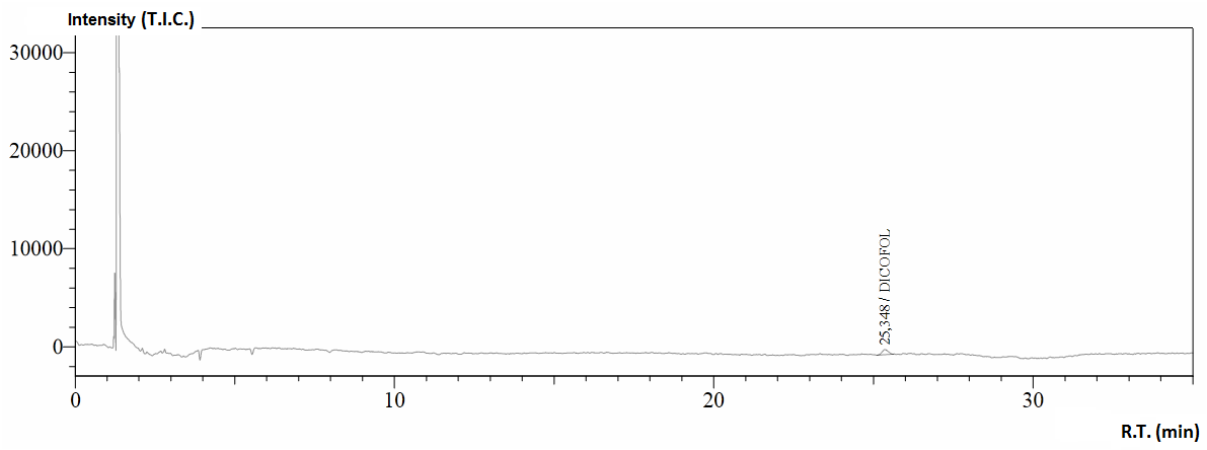

Figure 5: GC-ECD analysis of 0.005 mg kg^{-1} Dicofol, retention time 25.3 minutes.

The lower concentration quantified was 0.025 mg kg^{-1}, according to the chromatogram in Fig. 6.

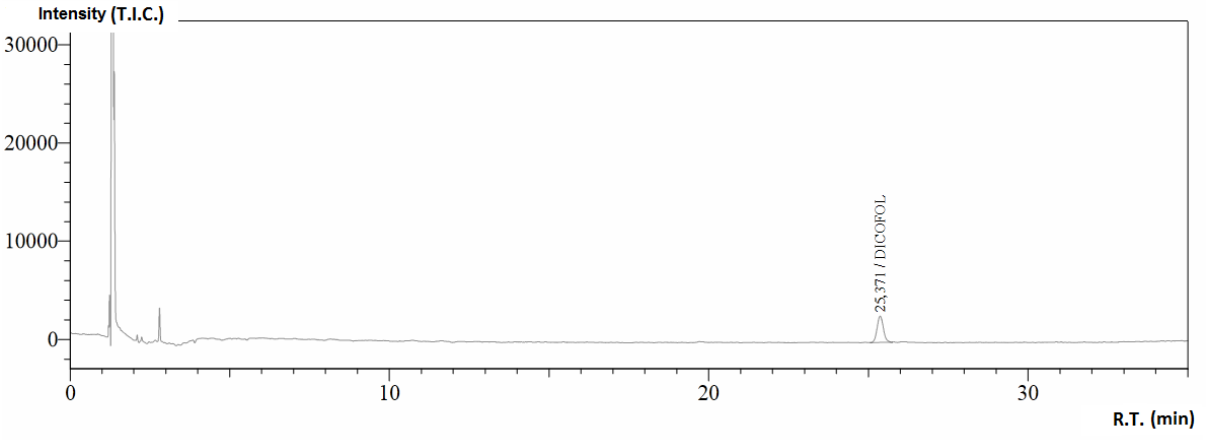

Figure 6: GC-ECD analysis of Dicofol 0.025 mg kg^{-1} Dicofol, retention time 25.3 minutes.

3.2 Calibration curve

The calibraton curve was done with five points. The mean area and their relative concentrations were processed with Excel (2010), where a dispersion graph was drawn, according to Fig. 7. The R^2 value is 0.9987.

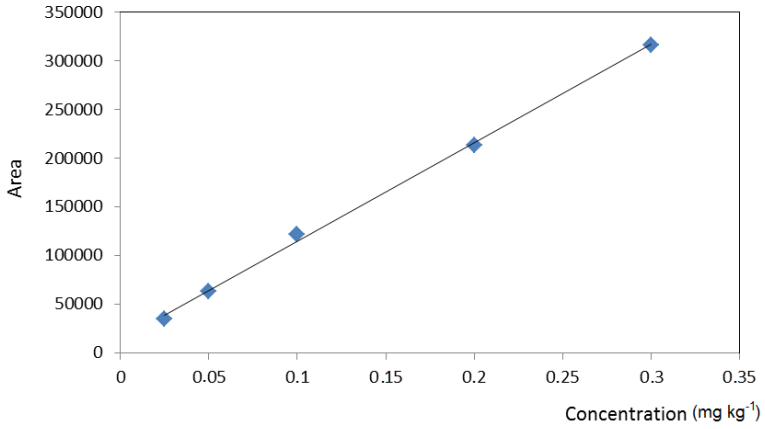

Figure 7: Calibration curve for Dicofol. (Excel 2010)

The samples were quantified using the following equation (Eq. 1):

$$y = 1.0120\ \ 10^{6}\ x + 1.3547\ \ 10^{4} \qquad (1)$$

In Which,
y = Area
x = Concentration in mg kg^{-1}
Angular coefficient = $1.0120\ \ 10^{6}$
Linear coefficient = $1.3547\ \ 10^{4}$

Table 2 presents the results of mean concentration an standard deviation for three injections of each sample.

Table 2. Results for Dicofol analysis in orange juice samples.

Results – DICOFOL			
Sample identification	**Mean concentration (mg kg^{-1})**	**Standard deviation (mg kg^{-1})**	**Recovery (%)**
Sample N	<LOD	-	101
Sample O	0.030	0.001	94
Sample Q	<LOD	-	105
Sample C	<LOD	-	148

Results obtained by calculation with Excel, 2010.

The Fig. 8, 9, 10 and 11 show the chromatograms of the samples.

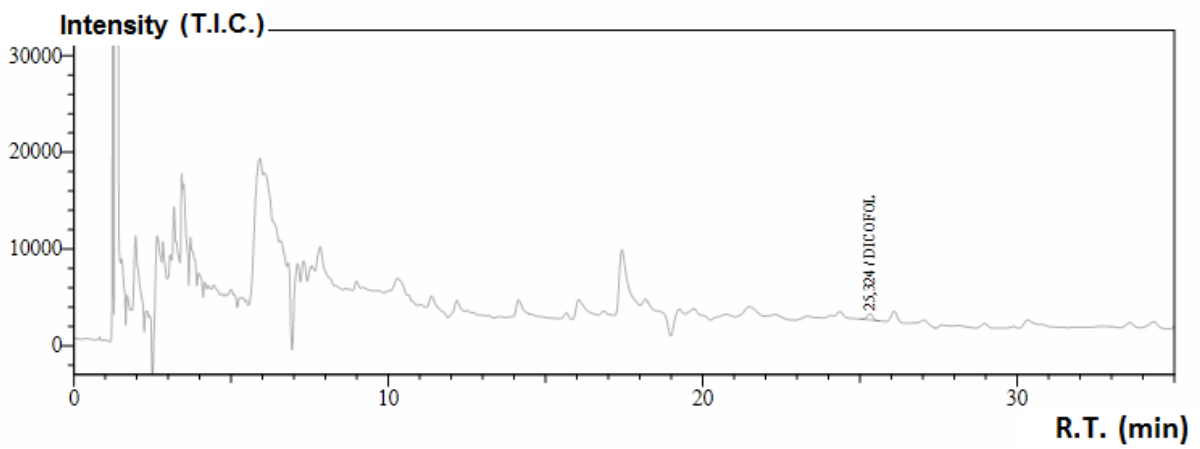

Figure 8: Sample N presented the retention time in 25.3 minutes (peak of Dicofol), with area less to the LOD.

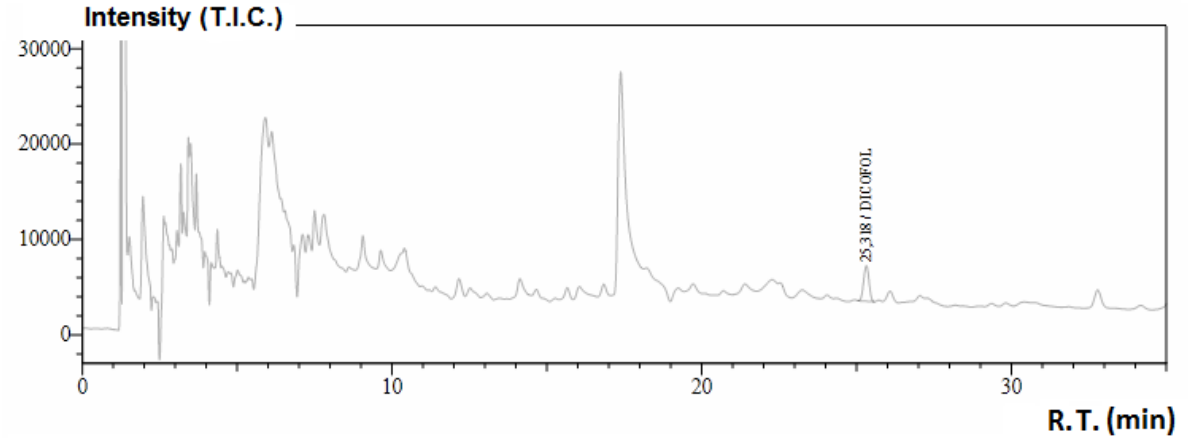

Figure 9: Sample O presented the retention time 25.3 minutes and it's observed a peak related to Dicofol.

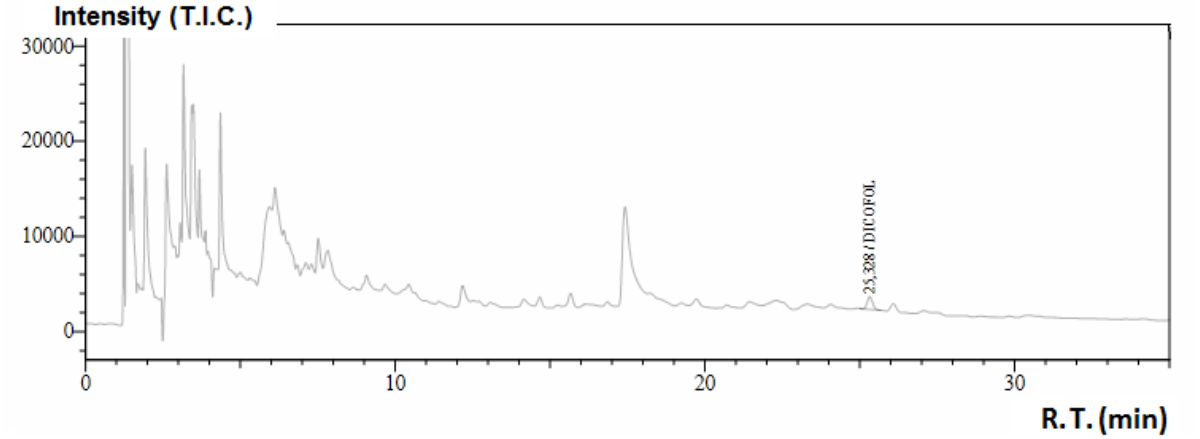

Figure 10: Sample Q presented the retention time 25.3 minutes and it's observed a peak related to Dicofol, with area less to the LOD.

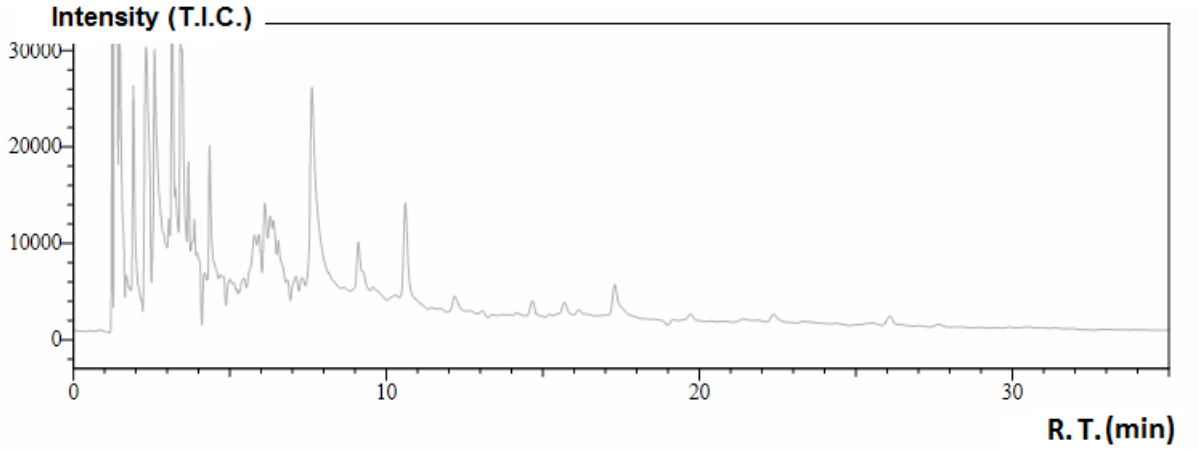

Figure 11: Sample C is not observed a peak related to Dicofol.

For samples N and Q it was found concentration values for Dicofol below the LOD (0.005 mg kg^{-1}). For sample C it was not detected any peak in the chromatogram.

Sample O presented concentration for Dicofol close to the first calibration point and, after the calculations considering mass and volume of sample the calculated concentration was 0.03 mg kg^{-1}.

In the QuEChERS extraction process, the final extracts had a light-yellow color. Observing the chromatograms, it can be noticed that at the beginning of the analysis there is a rise in the baseline and a series of peaks that are characteristic of the sample profile. As the analyte of interest was detected at the retention time 25.3 minutes, its separation is well defined and easily quantified.

The recovery of the standard in the samples was efficient (94-105) %. However, for the sample C, the recovery obtained was 148 %. Possibly, this occurred due to some contamination in the extraction process.

4. CONCLUSION

Among the four analyzed samples in this work, only the sample O showed concentration above the LOQ, with 0.03 mg kg^{-1} of Dicofol. When comparing this value with the MRL in *Codex Alimentarius* (0.1 mg kg^{-1}), the sample presents MRL below this limit. Therefore, all the analyzed samples comply with the regulations and are safe for human consumption.

The extraction technique chosen (QuEChERS) was easy to apply and fulfilled the requirements for orange juice matrix, resulting in a clear extract and with good recovery for 75 % of the analyzed samples.

The analytical technique (GC-ECD) showed to be efficient for the analysis of Dicofol, and it was possible the detection of peaks with excellent resolution for a concentration of 0.005 mg

kg^{-1} and quantification from 0.025 mg kg^{-1}. The ECD with ^{63}Ni nuclide has the best sensibility to the analyzed compound, and it is recommended for trace analysis of organochlorine compounds in citric foods matrices.

5. ACKNOWNLEGMENTS

To IPEN and IPT for all support, knowleadge and infrastructure used in this work.

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