Validation and Uncertainty of the method for multiresidue analysis of 35 pesticides in melon using Gas Chromatography Coupled to Quadrupole Mass Spectrometry (GC-QP/MS)

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Abstract. QuEChERS method and detection in GC/SQ-MS were validated for the analysis of 35 pesticides in melon. Validation parameters (selectivity, linearity, LOD, LOQ, accuracy and precision) were determined according ABNT NBR 14029:2005. The recoveries rates for all the pesticides studied were from 63% to 117% with relative standard deviation (RSD) lower than 15% in the concentration range of 0.05 - 0.20 mg/kg. The limit of quantification (LOQ) for most compounds were below the MRLs established in Brazil. The combined relative uncertainty ($U_c$) and expanded uncertainty ($U_e$) was determined using repetitivity, recovery and calibration curves data for each pesticide.

1. Introduction

Multiresidue analysis of complex matrices such as fruit requires constant modifications and optimizations methodologies and therefore need to be validated. Agencies like ANVISA and INMETRO, require validation of analytical methods as a key requirement in the accreditation for quality assurance and demonstration of technical competence.

According to ANVISA, validation should ensure through experimental studies that the method meets the requirements of analytical applications, ensuring the reliability of the results [1]. The analytical parameters normally determined for validation of methods of separation are: selectivity, linearity,
precision, accuracy, limit of detection, limit of quantification and robustness. One of the requirements of ABNT NBR 14029:2005, is the determination of the uncertainty associated with the results [2].

According EURACHEM the term uncertainty is “a parameter associated with the result of a measurement, which characterizes the dispersion of the values that could reasonably be attributed to the measurand [3]. In estimating the overall uncertainty, it may be necessary to take each source of uncertainty and treat it separately to obtain the contribution from that source. Each of the separate contributions to uncertainty is referred to as an uncertainty component. When expressed as a standard deviation, an uncertainty component is known as a standard uncertainty.

The gas chromatography coupled to quadruple mass spectrometers (GC-QP/MS) has been used for multiresidue analysis due to its good resolution, selectivity and sensibility expected given purpose, in addition to its versatility, featuring condition in different matrices [4]

Recently several articles related to multiresidue method validation in fruits has been published [5-6]. However, few articles report the determination of measurement uncertainty as parameter validation [7].

The work aims at validating and determining the uncertainty of multiresidue method in the matrix of melon and quantification by GC-QP/MS. The implementation of the method of analysis will expand the scope of laboratory analysis, allowing quality control of fruit, by monitoring the level of residues.

2. Materials and Methods

2.1. Chemicals, reagents and samples extraction
Certified standards for 35 pesticides studied were purchased from Sigma-Aldrich (Brazil) and Dr. Ehrenstorfer (Brazil). All the standards and reagents had purities exceeding 97.0%. Melon samples were analyzed based on QuEChERS method described by Anastassiades et al [8].

A blank matrix of the melon were prepared by adding spiking solution to blank extract, to produce a final concentration of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg.Kg\(^{-1}\).

2.2. Chemicals, reagents and samples extraction
It was used a gas chromatograph coupled to single quadrupole mass spectrometers (GC-SQ/MS, DSQII model, Thermo, USA), of the Fundação Núcleo de Tecnologia Industrial do Ceará - NUTEC. A Vertibond 50 m x 0.25 mm i.d. capillary column with a 0.10 µm film was used in the pesticide separation and helium as carrier gas at a constant flow of 1 mL.min\(^{-1}\). The temperature program was the following: initial temperature 100 °C held for 1 min, 15 °C.min\(^{-1}\) rate to 180 °C, then 4 °C.min\(^{-1}\) rate to 280 °C and held for 14 min. The injection temperature was 250°C, and 1 µL volume was injected in splitless mode (45.3 minutes). The mass spectrometer was operated in electron impact (EI) mode, ion source temperature 200°C, MS Transfer Line 270 °C, electron multiplier voltage 1295 V, scanning from m/z 50 to 500 at 2.0 s per scan; solvent delay 6.0 min.
Quantitative analysis was performed in the selected ion monitoring mode (SIM) based on the use of one ion target (T) and two or three ions qualifiers (Q1, Q2 and Q3).

2.3. Validation and Uncertainty
A blank matrix of the melon were prepared by adding spiking solution to blank extract, to produce a final concentration of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg.Kg$^{-1}$.

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated in the SIM mode analysis as the lowest concentration injected that yielded S/N ratio of 3 and 10, respectively.

Accuracy and precision of the method were tested with recovery experiments, performed with five replicates of blank samples spiked with pesticides at 0.05, 0.10 and 0.20 mg.kg$^{-1}$ and determination of relative standard deviation (RSD, %).

Uncertainty was determined for all of the pesticides, according to the procedures recommended by EURACHEM/CITAC Guide CG 4 [3]. The uncertainty of measurement was obtained at the level of 0.05 mg.Kg$^{-1}$ using validation data. Three sources of uncertainty were taken into account: uncertainty associated with precision ($u_1$), uncertainty associated with bias ($u_2$) and uncertainty associated with the calibration curve ($u_3$). The combined relative uncertainty ($U_c$) was calculated using the expression:

$$U_c = (u_1^2 + u_2^2 + u_3^2)^{1/2}$$ (1)

The expanded uncertainty ($U_e$) was obtained by multiplying $U_c$ by a coverage factor $k=2$ (confidence of 95%).

3. Results and Discussion

3.1. Method Validation

The standard matrix was used for determining the linearity range of pesticides studied. The correlation coefficients obtained were all above 0.99 and the linearity range from 0.02 to 0.75 mg Kg$^{-1}$ was considered satisfactory.

The LOD and LOQ values obtained ranged from 0.005 - 0.050 mg.Kg$^{-1}$. The limit of quantification (LOQ) for most compounds were below the MRLs established in Brazil.

The recoveries for all the pesticides studied were from 63% to 117% with relative standard deviation lower than 15%. According to ABNT NBR 14029:2005, the range of acceptable recovery level of concentration of 0.01 mg.kg$^{-1}$ is 60 - 115%, and 0.1 mg.kg$^{-1}$ is 80-110% [2]. The ANVISA recommended recovery percentages of 70 - 120% (> 0.01 - 0.1 mg.kg$^{-1}$) and RSD ≤ 20% [1].

The QuEChERS method was used by Barakat et al, in the extraction of 36 pesticides in honey samples, using the GC-ECD and GC-NPD systems. Afforded recovery percentage between 70 and 120% for RSD ≤ 22% [9]. Nguyen et al, achieved satisfactory recoveries (80% to 115%) for 107 pesticides in cabbage and radish [10]. Hernández-Borges et al, using the method QuEChERS and GC-NPD for the determination of 11 pesticides in banana obtained recovery percentages between 67 and 118% with RSD ≤ 16% [11].

3.2. Measurement of uncertainty analyses

Uncertainty of measurement represents a quantitative indicator of the reliability of the analytical results, expressed as a range which is estimated to be the real value, usually associated with a confidence level [3]. In an analytical procedure, the uncertainty about the result may arise from many possible sources, including sampling, matrix effects, environmental conditions, glassware, method of measurement and random variation. In this work, the combined relative uncertainty ($U_c$) and expanded uncertainty ($U_e$) was determined using repetitivity, recovery and calibration curves data for each pesticide. The $U_c$ and $U_e$ values ranged from 4.0 - 18.1% and 7.9 - 36.1%, respectively. On the
basis of the expanded uncertainties values, the pesticides could be classified into three groups: group I \((Ue < 10\%)\), group II \((Ue: 10-20\%)\) and group III \((Ue > 20\%)\). The results are showed in Figure 1.

About 74\% of the pesticides analyzed showed \(Ue\) ranging from 10-20\% (Group II). The pesticides chlorpyrifos, pyraclostrobin, deltamethrin and azoxystrobin had low uncertainty values \((Ue < 10\%)\) and were classified in Group I. \(Ue\) values considered high \((>20\%)\) were observed in 14\% of pesticides. Chloroneb and metalaxyl showed \(Ue > 30\%\).

![Figure 1- Classification of pesticides into groups based on the values of expanded uncertainty (Ue) group I (Ue <10%), group II (Ue:10-20%) and group III (Ue >20%).](image)

The repeatability was the largest contribution to the measurement uncertainty. Other sources, such as purity of the reference standard feature small contribution in uncertainty values, not exerting considerable influence on the final value.

Frenich et al used LC-MS-MS to validate a method for the analysis of 31 multiclass pesticide residues. The uncertainty associated to the analytical method was lower than 23\% for all compounds tested [11]. Kanrar et al obtained measurement uncertainty of less than 20\% of method validation of 42 pesticide residues in tea [12].

**Conclusion**

The proposed multiresidue method was successfully validated and can be applied to monitoring of 35 pesticides in melon. The method offers low measurement uncertainty \((\leq 20\%)\), indicating the adequacy of the requirements by accrediting agencies. The repeatability was the largest contribution to the measurement uncertainty.

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