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Pesticides Analysis in Beans by Gas Chromatography Couplet with Tandem Mass Spectrometry

Mizutani G* and Bustillos O
Centro de Química e Meio Ambiente, Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil

***Corresponding author:** Mizutani G, Centro de Química e Meio Ambiente, Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil

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Abstract

Beans are part of the basic diet alimentation for Brazilian population, as they gather proteins, carbohydrates, vitamins, mineral salts, fibers, amino acids and essential nutrients such as iron and calcium, being a complete food that can be compared with the amount of protein that the meat has. Considering the beans world production, in development countries represent almost 50%, being that Myanmar, India and Brazil the top three position. The use of pesticides is widely spread in these countries to reduce agricultural losses due to pests that interfere with grain production. Therefore the risk that could be generated from foods pesticides residues makes their analyses of quantification mandatory. The purpose of this work was to develop an analytical method to quantitatively characterize fungicides pesticides residues, flutriafol, procymidone and tebuconazole that were used to angular spot control, anthracnose, rust and alternaria spot, white mold fungi, present in beans, by means of gas chromatography coupled with a triple quadrupole mass spectrometer. Samples of beans, *Phaseolus vulgaris L*, types white, black, string and *Vigna angularis*, type adzuki, had been bought in grains store and supermarkets at metropolitan São Paulo city. The validation of analytical method was explored for sensitivity, selectivity, precision. The extraction procedure was performed in two different forms, QuEChERS, and solid-liquid extraction with low temperature. Through this methodology, reached below the maximum limit allowed by Brazilian law 0.5mgkg^{-1} for procymidone and 0.1mgkg^{-1} for flutriafol and tebuconazole. Several samples of four types of beans were tested and all of them had procymidone identified and 7% of samples higher than the law limit.

Keywords: Beans; GCMSMS; Pesticides

Introduction

In Brazil, beans are the most popular food for general population, they have nutrients and energy that work in health prevention. In their composition there are proteins, carbohydrates, vitamins, mineral salts, fibers and amino acids [1].

Grains producers have been using several pesticides in this vegetable culture to preserve and improve the crop. The fungicides utilization has a function of preventing plant tissues infection by phytopathogenic fungi, currently we can find other concepts such as chemical compounds that are used to control diseases that are caused by fungi, bacteria and algae, in some cases they do not eliminate fungi, but temporarily inhibit spore germination [2].

The procymidone is using in mold-white fungi in a concentration of 500gkg^{-1} and acts to inhibit the growth of micelles, in the protection, cure [3,4].

The flutriafol and tebuconazole are fungicides that doing inhibit the synthesis of sterols that act in the formation and selectivity of the plasma membrane are used to control angular stain, anthracnose, rust and alternaria stain [3,4].

Fruits, vegetables, cereals are the most matrices that could find some pesticides residues in different classes and method development for this compounds are very important [5].

For these pesticides analyses several extraction are being used to aim these analytes. Acetonitrile is the best solvent to extract the samples because it has the best interaction with the analytes [6].

Therefore sample preparation using the QuEChERS multiresidues method, starts with the sample grind to get the most surface area to be in solvent contact, add magnesium sulfate, sodium chloride or sodium acetate, drying agents and clean up processes [7,8].

Other extraction technique used was solid-liquid with low temperature, in this extraction the sample, liquid or solid, in contact with solvent less dense than water and the less melting point less than 20ºC negative, after shaking, rest in freezer about 16 hours, the water phase will be freeze and the organic phase will be liquid, this part has to be transfer to a vial and chromatography analyses [9,10].

This paper developed a method to characterize and quantify the fungicides that is present in vegetable with high protein content, beans specifically, that used a gas chromatography with mass spectrometry triple quadrupole TANDEM.

Methods and Materials

Materials and equipment

The following materials were used in this work:

- Falcon tube 10mL and 50mL;
- Analytical standards, flutriafol, procymidone, tebuconazole;

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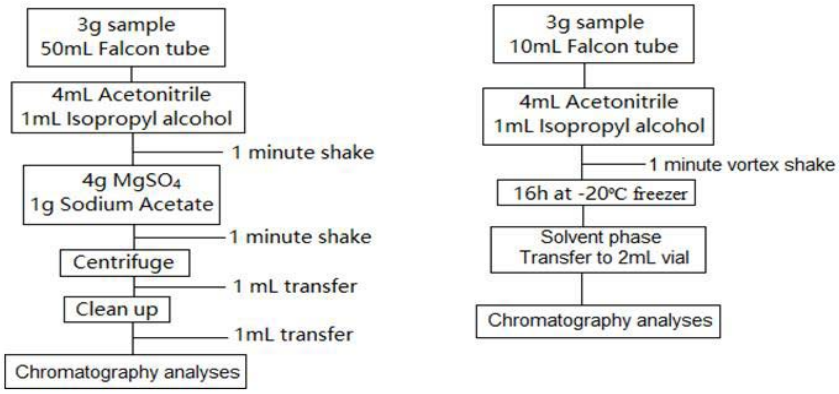

Figure 1: Extraction flowchart.

- Acetronitrile HPLC grade, isopropyl alcohol HPLC grade and ultrapure water;
- Vortex;
- Low temperature freezer -20ºC;
- Blender;
- Gas chromatography coupled with Tandem mass spectrometry Bruker-EVOQ.

Method

Extraction: For extraction it was used a two different methods: QuEChERS, the most analytical extraction for pesticides, was used sample crushed, 3g, extraction salt, magnesium sulfate, 4g, and sodium acetate, 1g, with organic solvent, 4mL acetonitrile and 1mL isopropyl alcohol, shake 1 minute, centrifuge transfer 1mL to and clean up vial 150mg magnesium sulfate and 50mg PSA, transfer to 2mL vial and chromatography analyses, the second method solid-liquid with low temperature, was used a sample crushed, 3g, in contact with organic solvent less dense than water, 4mL acetonitrile and 1mL isopropyl alcohol, and fusion point less than 20ºC negative, the organic phase was liquid, transfer to 2mL vial and chromatography analyses, flowchart should be observe in Figure 1.

Analytical method: For analytical method it was used an instrumental analytical, gas chromatograph coupled with Tandem mass spectrometry equipment. It was developed method to separate the analytes. This method had a good sensitivity, accuracy and precision to fungicides compounds. All parameters of this analytical method can be found in Table 1 and 2.

Table 1: Chromatography method.

Auto sampler	1µL	
Injector	250ºC	
Flow	1mLmin^{-1} splitless 0.80min	
Carrier Gas	Helium	
Column	5MS - 30m x 0.25mm x 0.25µm	
Oven		
Rate	**Temperature**	**Hold**
Initial	100ºC	0min
20ºC min^{-1}	220ºC	3min
40ºC min^{-1}	280ºC	4min

Table 2: Mass spectrometry method.

Source	250ºC
Transfer line	230ºC
MS mode	MRM - Multiple Reaction Monitoring
Diuron	161 > 90 (10eV)
Flutriafol	219 > 164.70 (10eV)
Procymidone	96 > 67 (10eV)
Tebuconazole	250 > 125 (10eV)

Validation

The analytical method validation has been processed with efficiency; the following parameters were evaluated to suitability guarantee.

These are selectivity, linearity, work range, detection limit, quantitation limit, recovery and accuracy. All validation steps add compound in matrix and follow the INMETRO DOQ-CGCRE-008, Brazilian validation document [11].

Selectivity

An analytical method to quantify the aim analyte in presence with other analytes or interference material was established. To identify the selectivity, standard injection had been done, to evaluate, retention time, fragmentation and column analytes separation. Full scan mode with extraction ions and four multiple reaction monitoring, MRM were done, these are shown in Figure 2.

Linearity and work range

Linearity is an analytical procedure that can produce results proportionality to a sample analyte concentration. Work range is the range between the highest and lowest sample concentration, (10ugkg^{-1}, 25ugkg^{-1}, 50ugkg^{-1}, 75ugkg^{-1}, 100ugkg^{-1}, 250ugkg^{-1}, 500ugkg^{-1}) that it has the method with an acceptable precision, accuracy and linearity. In Figure 3 it can see the linearity for four analytical compounds.

Detection and quantitation limit

Detection limit is the lowest concentration that can be found but not necessarily quantified. For this test all spectra compound has a

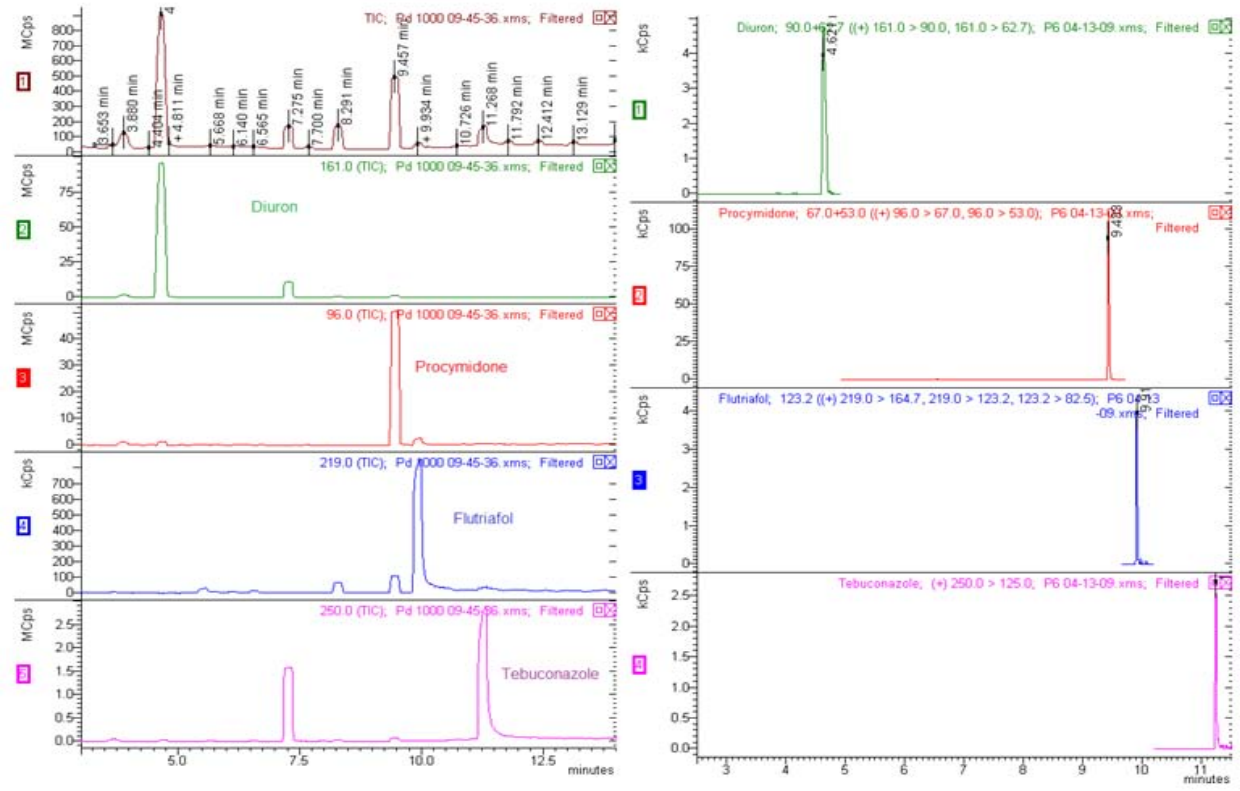

Figure 2: Full scan, left and MRM, right, analyses.

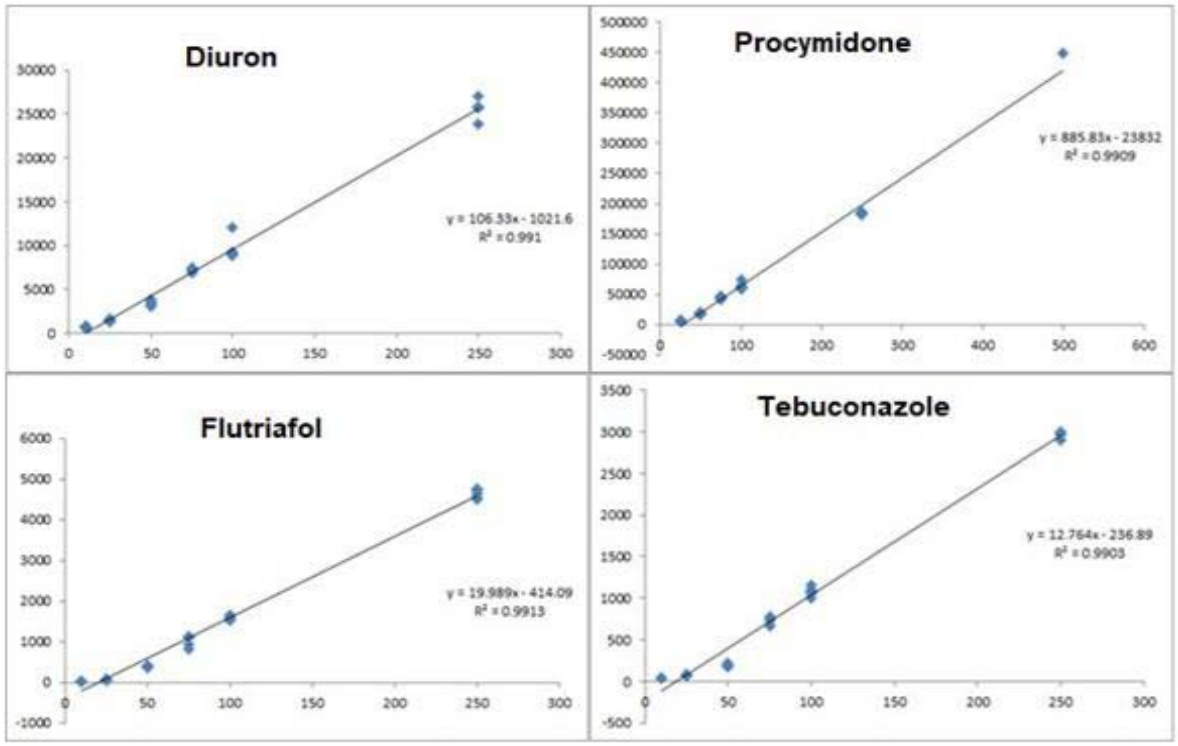

Figure 3: Calibration curve.

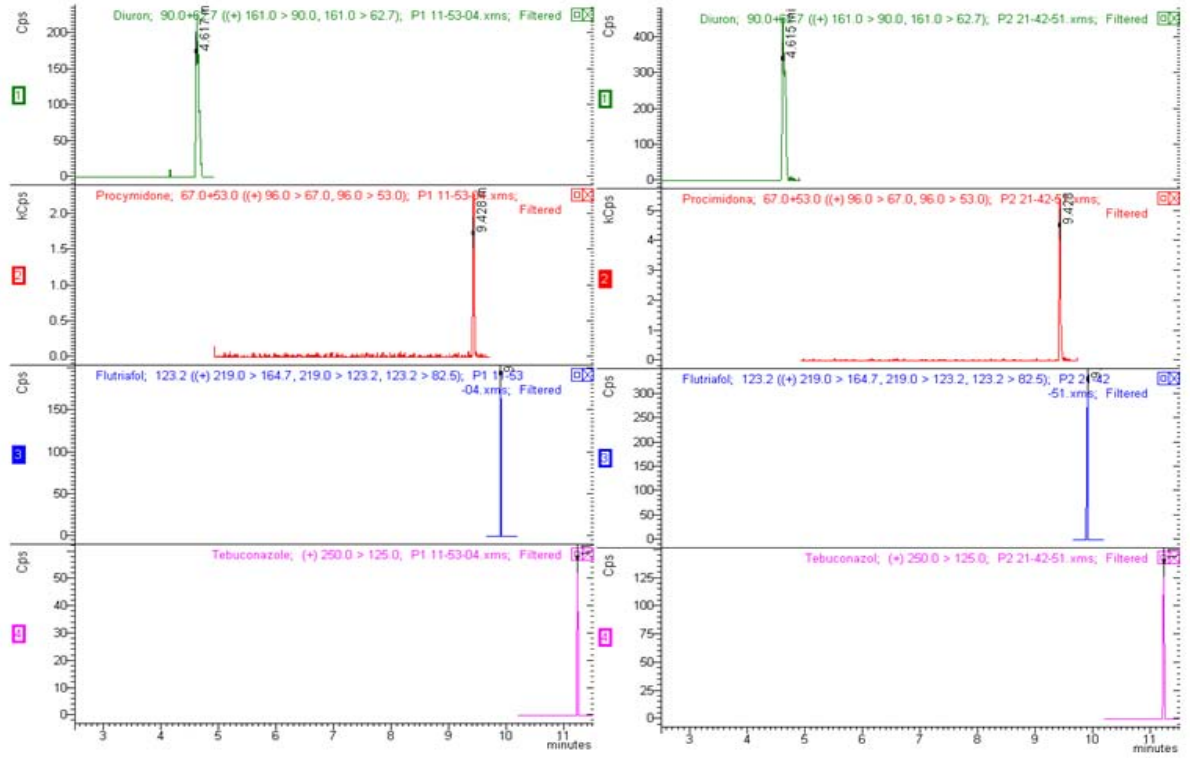

Figure 4: Detection limit, left and Quantitation limit, right.

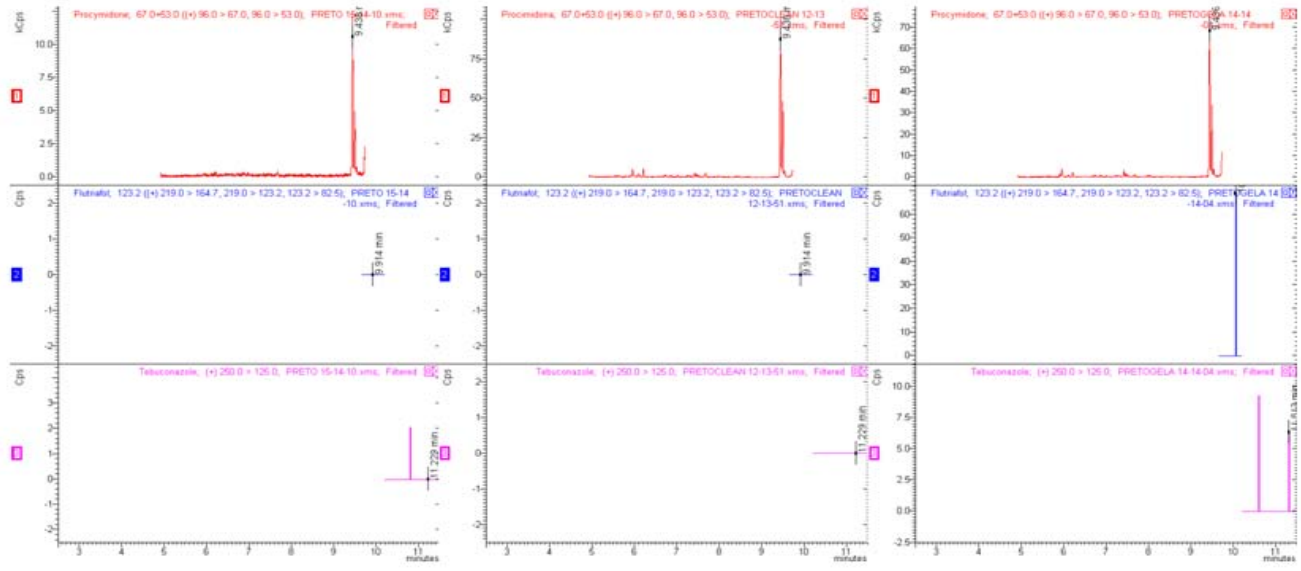

Figure 5: Sample spectrum, left without cleanup, middle with cleanup, right low temperature.

lowest concentration 10ugkg^{-1} detectable.

Quantitation limit is the less sample quantity that can be quantified with precision and accuracy. For quantitation limit, four compounds used 25ugkg^{-1}. Figure 4 shows a spectrum for detection limit, in the left hand and quantitation limit, in the right hand.

Recovery

Recovery is estimated by fortified sample analysis with known compound and three different concentrations, low, 25ugkg^{-1}, medium, 75ugkg^{-1} and high, 250ugkg^{-1}, the recovery range is between 80-110. It was used two different compounds to evaluated recovery, Diuron and Tebuconazole standard in solvent, observe the recovery

Table 3: Diuron recovery.

Sample	Amount	Reference	Recovery	Sample	Amount	Reference	Recovery	Sample	Amount	Reference	Recovery
1	24.915	25	99.66	**1**	79.865	75	106.486	**1**	251.538	250	100.615
2	24.117	25	96.468	**2**	73.68	75	98.24	**2**	241.158	250	96.463
3	22.005	25	88.02	**3**	75.573	75	100.764	**3**	247.505	250	99.002
4	23.789	25	95.156	**4**	73.003	75	97.337	**4**	244.964	250	97.985
5	22.809	25	91.236	**5**	72.969	75	97.292	**5**	243.366	250	97.346
6	22.933	25	91.732	**6**	78.727	75	104.969	**6**	241.52	250	96.608
7	22.242	25	88.968	**7**	72.842	75	97.123	**7**	233.069	250	93.227
8	20.983	25	83.932	**8**	70.818	75	94.424	**8**	245.562	250	98.224
9	21.965	25	87.86	**9**	67.38	75	89.84	**9**	244.704	250	97.881
10	22.064	25	88.256	**10**	73.77	75	98.36	**10**	246.558	250	98.623
Average	22.782			**Average**	73.862			**Average**	243.994		
SD	1.19			**SD**	3.6			**SD**	4.87		
RSD(%)	5.21			**RSD(%)**	4.87			**RSD(%)**	2		

Table 4: Tebuconazole recovery.

Sample	Amount	Reference	Recovery	Sample	Amount	Reference	Recovery	Sample	Amount	Reference	Recovery
1	27.017	25	108.068	**1**	73.47	75	97.96	**1**	236.964	250	94.785
2	22.18	25	88.72	**2**	69.259	75	92.345	**2**	252.761	250	101.104
3	22.711	25	90.844	**3**	78.465	75	104.62	**3**	252.986	250	101.194
4	22.265	25	89.06	**4**	74.692	75	99.589	**4**	238.172	250	95.268
5	27.209	25	108.836	**5**	82.358	75	109.81	**5**	248.208	250	99.283
6	27.036	25	108.144	**6**	75.666	75	100.888	**6**	252.419	250	100.967
7	26.548	25	106.192	**7**	75.92	75	101.226	**7**	252.308	250	100.923
8	26.634	25	106.536	**8**	71.885	75	958.466	**8**	254.679	250	101.871
9	24.045	25	96.18	**9**	83.509	75	111.345	**9**	240.578	250	96.231
10	21.121	25	84.484	**10**	78.16	75	104.213	**10**	240.134	250	96.053
Average	24.676			**Average**	76.338			**Average**	246.92		
SD	2.44			**SD**	4.43			**SD**	7.1		
RSD(%)	9.9			**RSD(%)**	5.81			**RSD(%)**	2.88		

in Table 3 and Table 4.

Accuracy

In the accuracy was not more than 20% and the standard deviation relative was not more than 5%. There are three most common evaluations: repeatability, intermediate precision and reproducibility, usually expressed by standard deviation and coefficient of variation. For this validation it was used repeatability which means that this procedure needs the same requirements, such as operator, preparation conditions, systems, and place. The compounds were added the compounds in the matrix and used different range concentration, low, 25ugkg^{-1}, medium, 50ugkg^{-1} and high 250ugkg^{-1} (Table 5-7).

Results and Discussion

The analytical method validations were satisfactory for selectivity, sensitivity, linearity and accuracy. Two different extractions were done, for QuEChERS extraction we made a comparative between clean-up and without clean-up. The results showed that cleanup was very effective to matrix effect and reduced the signal to noise chromatogram protein and showed better results than without cleanup, Table 8. The solid- liquid extraction at low temperature had different results for all types of beans, these results may have occurred due to bad interaction of the contact surface or bad organic solvent interaction (Table 9). Therefore, in this comparative extraction method low temperature was not efficient to these fungicides' compounds.

All samples had showed procymidone as identified and their quantitative results are shown at Table 10. Nevertheless the most procymidone samples results are below the Brazilian law regulation limit 0.5mgkg^{-1} and that means that it is good for consumption but in 7% in all sample showed a lit bit higher than law regulation limit. Flutriafol and tebucanozole don't appear in spectrum results or above the detection limit (Figure 5).

Conclusion

The methodology developed in this work shows a good agreement due to validation method for linearity, sensibility, accuracy, detection

Table 5: Flutriafol low precision.

	Sample	Concentration	Referencie Concentration	Accuracy (%)
Flutriafol	White beans			
	1	23.671	25	-5.316
	2	23.614	25	-5.544
	3	23.764	25	-4.944
	4	26.196	25	4.784
	5	24.202	25	-3.192
	Average	24.289		
	SD	1.09		
	RSD(%)	4.49		
	Sample	Concentration	Referencie Concentration	Accuracy (%)
Flutriafol	Adzuki beans			
	1	23.753	25,000	-4,988
	2	24.216	25,000	-3,136
	3	23.171	25,000	-7,316
	4	23.528	25,000	-5,888
	5	23.103	25,000	-7,588
	Average	23.554		
	SD	0.46		
	RSD(%)	1.93		

Table 6: Diuron medium precision.

	Sample	Concentration	Referencie Concentration	Accuracy (%)
Diuron	String beans			
	1	58.517	50	17.034
	2	58.606	50	17.212
	3	57.982	50	15.964
	4	54.312	50	8.624
	5	56.627	50	13.254
	Average	57.209		
	SD	1.8		
	RSD(%)	3.15		
	Sample	Concentration	Referencie Concentration	Accuracy (%)
Diuron	White beans			
	1	49.924	50	-0.152
	2	56.291	50	12.582
	3	54.79	50	9.58
	4	55.175	50	10.35
	5	54.15	50	8.3
	Average	54.066		
	SD	2.44		
	RSD(%)	4.52		

Table 7: Tebuconazole high precision.

	Sample	Concentration	Referencie Concentration	Accuracy (%)
Tebuconazole	White beans			
	1	228.017	250	-8.793
	2	244.589	250	-2.164
	3	229.1	250	-8.36
	4	247.478	250	-1.009
	5	234.857	250	-6.057
	Average	236.808		
	SD	8.87		
	RSD(%)	3.75		
	Sample	Concentration	Referencie Concentration	Accuracy (%)
Tebuconazole	Adzuki beans			
	1	248.837	250	-0.4652
	2	259.327	250	3.731
	3	239.374	250	-4.25
	4	231.291	250	-7.484
	5	255.876	250	2.35
	Average	246.941		
	SD	11.61		
	RSD(%)	4.7		

Table 8: QuEChERS comparations results.

		Beans				
		Black	White	String	Adzuki	Unit
QuEChERS	Without cleanup	0	0	0	0	μgkg^{-1}
		0	0	0	0	
		0	0	0	0	
		0	0	0	0	
		0	0	0	0	
	With cleanup	24.171	24.162	23.268	24.546	μgkg^{-1}
		24.423	24.072	25.268	24.233	
		24.28	23.561	24.83	25.963	
		23.422	23.932	23.814	26.6	
		23.823	25.142	22.896	24.627	

Table 9: Low temperature extraction results.

	Beans				
	Black	White	String	Adzuki	Unit
Low temperature	145.117	77.314	203.524	103.577	μgkg^{-1}
	143.115	77.61	199.542	102.983	
	43.567	31.932	25.024	0.000	
	40.059	45.41	70.903	30.968	
	35.888	49.25	59.902	0.000	

and quantitation limit.

Two different extractions were tested and it was observed that low temperature extraction has a disadvantage, a long time inside low temperature freezer, about 15 hours, to freeze the aqueous phase but was not efficient for these fungicides compounds, but it is not necessarily useless for other pesticides compounds.

Table 10: Sample Results.

Sample	Feijão				Unit
	Black	White	String	Adzuki	
1	188.562	376.87	39.799	28.038	
2	81.485	47.794	37.58	46.64	
3	76.05	53.36	41.263	48.008	
4	80.736	47.213	36.133	46.229	
5	80.422	37.362	37.039	49.239	
6	77.025	29.606	37.458	50.501	
7	72.305	28.769	35.849	47.078	
8	295.22	40.293	259.189	31.537	
9	228.267	39.446	264.227	35.264	
10	143.115	40.36	265.558	35.286	
11	43.567	39.164	261.514	35.319	
12	128.507	39.388	274.735	31.957	
13	85.193	39.66	43.386	32.938	
14	83.439	216.96	35.667	31.284	
15	76.419	235.209	35.978	269.502	
16	67.355	252.365	33.286	273.641	µg kg^{-1}
17	64.969	214.37	33.251	264.055	
18	318.243	198.962	31.454	258.239	
19	229.906	203.131	30.278	263.66	
20	230.778	183.936	28.689	427.029	
21	237.54	296.371	34.841	411.554	
22	492.752	286.666	36.896	625.149	
23	515.174	267.477	33.919	283.468	
24	256.627	267.268	34.278	353.411	
25	276.036	260.405	33.076	513.987	
26	467.731	286.363	31.281	311.317	
27	632.52	378.321	584.215	550.785	
28	287.996	670.378	484.459	188.446	
29	126.227	188.18	505.939	174.998	
30	137.286	618.594	295.423	265.352	
31	224.257	415.396	98.444	52.011	

In QuEChERS extraction showed that the clean-up extraction was efficient to prevent matrix effect, the signal to noise chromatogram protein is reduced and better results were achieved, but in disadvantage some difficult to buy a extraction salt and a abruptly agitation about 1 minute.

We were analyzed about 30 samples and the fungicides results in the beans stood below the regulation limit, which means that they are good for consumption except for 2 sample in 30, that a lit bit higher than regulation limit, that maybe bought when the store recently receive, this proves that we have a fungicides degradation by time of exposure.

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