Validation of the AOAC 2007.01 method for pesticides residues analysis in oranges and mandarins in LATU

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All over the world pesticides are used to control plagues on excessively worked lands. However, although they could be necessary, these can have negative effects on population.
Clearly, this is the situation in citrus production, so fruit growers and exporters need a laboratory available with validated and accredited methods for the corresponding analyses.
In Uruguay, the European Union is one of the most important buyers for citrus fruits. Consequently, as an answer to maximum residues limits demanded by UE legislation, we present in this work an analytical method developed in order to satisfy these requirements.
Based on the AOAC 2007.01 official method [1], we validated a methodology using GC-μECD and HPLC-FLD with GC-MS confirmation for Malathion, Chlorpyriphos, Captan, Folpet, Prochloraz, Imazalil, Thiabendazole (TBZ) and o-Phenylphenol (OPP)

EQUIPMENT:

HPLC Agilent 1100 with:
Fluorescence Detector G1321A
Automatic Injector Agilent 1313A
Column: C18 15 cm x 4.6 mm x 5 μm and C18 15 cm x 4.6 mm x 3 μm

HRGC Agilent 6890 with:
μECD Agilent
Automatic Injector Agilent 7683B
Column: DB-XLB 30 m x 250 μm x 0.25 μm
Electronic Pressure control

HRGC Agilent 7890A with:
LRMS Agilent 5975C Inert
Automatic Injector Agilent 7683B
Column: DB-XLB 30 m x 250 μm x 0.25 μm
Electronic Pressure control

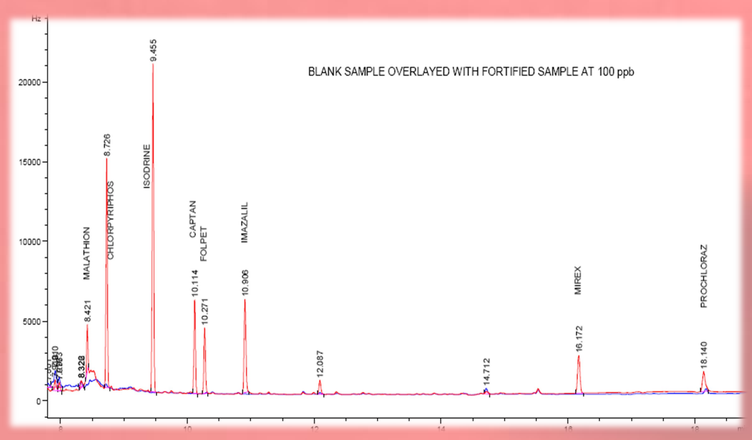
EXPERIMENTAL:

Sample is trated as in [1] and injected in GC-uECD for Malathion, Chlorpyriphos,Captan, Folpet, Prochloraz and Imazalil and in HPLC-FLD for Thiabendazole and o-Phenylphenol. The final extract with no change of solvent is used.
Due to matrix effects the calibration curves are prepared in blank matrix except for o-Phenylphenol.

HPLC-FLD analysis
Injection volume: 15 μl
mobile phase: H_2O pH=2,5:ACN (50:50) for OPP
H_2O 1.5% NH_3:ACN (80:20) for TBZ
flow: 1.5 ml/min for OPP and 0.8 ml/min for TBZ
oven temperature: 25 ºC
FLD operating at 285-365 nm

HRGC-uECD analysis
Injection volume: 2 μl (splitless)
constant flow: 1.2 ml/min
oven temperature: from 40ºC to 180 ºC (50 ºC/min)
from 180 ºC (10`) to 230 ºC (10 ºC/min)
from 230 ºC to 280 ºC (35 ºC)
μECD temperature: 300ºC

HRGC-LRMS analysis
Injection volume: 1.2 μl (splitless)
constant flow: 0.7 ml/min
oven temperature: the same used in HRGC-uECD analysis
Ionization source: EI 70 eV 250 ºC
Quadrupole temperature: 150 ºC
Interface temperature: 250 ºC



VALIDATION DATA:

1-ACCURACY:

Note: We measure trueness and precision of Isodrine in order to use it as an internal standard to control the process

a- **Trueness**: we use as a measure of trueness the recovery from spiked blank samples (samples of oranges and mandarins that we confirmed they do not have detected levels of the pesticides of interest)

For recovery we spiked the blank sample at different levels with each pesticide solution prepared in acetonitrile with 1% acetic acid. One level of spike is the MRL, and the others are above and below it. Acceptance criteria for recovery: 70-120% [2]

Malathion : 88.3 – 111.3% (60-100 μg/Kg)
Chlorpyriphos: 72.4 – 90.7% (50-800 μg/Kg)
Captan: 72.2 – 95.0% (50-100 μg/Kg)
Folpet: 71.4 – 97.6% (50-100 μg/Kg)
Imazalil: 76.0 – 105% (100-10000 μg/Kg)
Prochloraz: 84.2 – 93.7% (100- 20000 μg/Kg)
O-Phenylphenol: 73.0 – 110.8% (25-10000 μg/Kg)
Thiabendazole: 65.8 – 130.9% (60–10000 μg/Kg)
Isodrine: 74.1 – 98.7% (20-100 μg/Kg)

b- **Precision:** For repeatability we analyzed in the same day a minimum or 5 replicates with the same analyst. For intermediate reproducibility we repeated the analysis during different days. Acceptance criteria RSD< 20 for levels above and equal the LOQ [2].

Repeatability:
Malathion: RSDr= 4.0-9.9%
Chlorpyriphos : RSDr= 3.7-14.8%
Captan : RSDr= 1.9-11%
Folpet : RSDr= 1.8-8.7%
Imazalil : RSDr= 2.8-9.2%
Prochloraz : RSDr= 4.0-16.3%
O-Phenylphenol : RSDr= 2.9-9.8%
Thiabendazole : RSDr= 1.8-13%
Isodrine : RSDr= 2.9-14.7%

Intermediate reproducibility :
Malathion:100 μg/Kg $iRSD_R$= 18.4% n=3
Chlorpyriphos : 100 μg/Kg $iRSD_R$ = 16.9% n=4
Captan : 100 μg/Kg $iRSD_R$ = 16.7% n=3
Folpet : 100 μg/Kg $iRSD_R$ = 2.4% n=2
Imazalil : 10000μg/Kg $iRSD_R$ =14.5% n=2
Prochloraz : 10000μg/Kg $iRSD_R$ = 18.8% n=2
O-Phenylphenol : 100 μg/Kg $iRSD_R$ = 12.9% n=4
Thiabendazole : 5000 μg/Kg $iRSD_R$ = 3,3% n= 2
Isodrine : 100 μg/Kg $iRSD_R$ = 7.8% n=5

2-CALIBRATION CURVE/LINEARITY:
Note: the amount specified is expressed in ug/Kg in sample. For GC quantitation we used Mirex as internal standard.

Malathion: 5-3500 μg/Kg r^2=0.999
Chlorpyriphos :5-3500 μg/Kg r^2=0.999
Captan: 5-3500 μg/Kg r^2= 0.994
Folpet: 5-3500 μg/Kg r^2= 0.995
Prochloraz: 5-3500 μg/Kg r^2= 0.999
Imazalil: 5-3500 μg/Kg r^2= 0.999
Thiabendazole:100-2500 μg/Kg r^2=0.998
O-Phenylphenol: 5-3500 μg/Kg r^2= 0.998
Isodrine: 4-3000 μg/Kg r^2= 0.999

3-DETECTION LIMIT (LOD):
To measure LOD we spike a minimum or 5 replicates of blank samples. Acceptance criteria signal to noise ratio *>3*
Malathion: 20 ug/Kg
Clorpiriphos: 20 ug/Kg
Captan: 20 ug/Kg
Folpet: 20 ug/Kg
Imazalil: 50 ug/Kg
Prochloraz: 50 ug/Kg
o-Phenylphenol: 10 ug/Kg
Thiabendazole: 40 ug/Kg

4-QUANTITATION LIMIT (LOQ) :
To measure LOQ we spike a minimum or 5 replicates of blank samples. Acceptance criteria: mean of recovery 70-120% with RSD% ≤ *20%* [2] and signal to noise ratio *>10 .*
Malathion: 60 ug/Kg
Clorpiriphos: 50 ug/Kg
Captan: 50 ug/Kg
Folpet: 50 ug/Kg
Imazalil: 100 ug/Kg
Prochloraz: 100 ug/Kg
o-Phenylphenol: 25ug/Kg
Thiabendazole: 100 ug/Kg

5-SPECIFICITY:
Blank samples analyzed in the batches show no interferences

6-UNCERTAINTY: Expanded uncertainty, calculated using sum of squares of type A and B components of uncertainty with a probability of 95% k=2 : 20%

CONCLUSIONS:

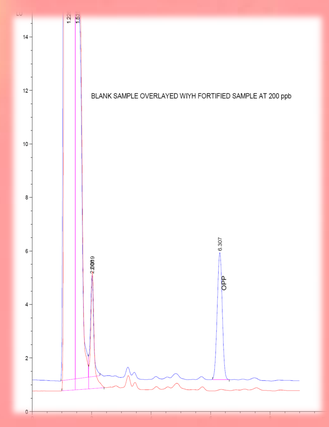
•The results show that FLD for HPLC and uECD for GC could be used with good results for routine application of QuEChERS method.

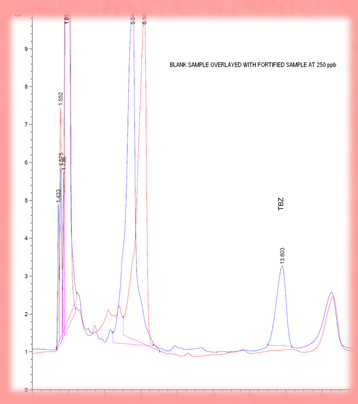
*The analysis presented here is sensitive, accurate and useful for routine analysis.

*We continued working in TBZ recovery in order to improve the results

BIBLIOGRAPHY:

[1]-Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. AOAC Official Method 2007.01, 2007
[2]- SANCO/10684/2009. Method Validation and Quality Control Procedures for Pesticides Residues Analysis in Food and Feed.





ACKNOWLEDGMENTS:

Departamento de Desarrollo de Métodos Analíticos-LATU, for their contributions to this work. This work is part of the final experimental work of L. Alcarraz to get the Quimica Farmacéutica degree from Facultad de Quimica UdelaR