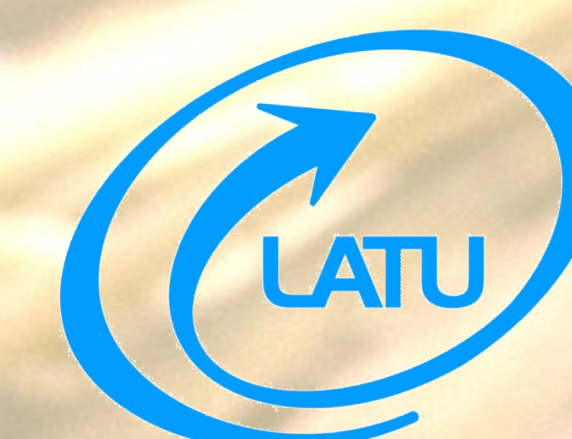




# VALIDATION OF THE DETERMINATION OF AMITRAZ, FLUVALINATE AND COUMAPHOS IN HONEY.



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## Introduction

According to the current legislation and requirements of honey-buyer countries, Uruguay tests biological residues in honey and other beehive products. The determination of this biological residues was validated based on the limits set by European regulations for each kind of residues including amitraz, fluvalinate and coumaphos. The method consists on the extraction of pesticides with a mixture of hexane:isopropanol followed by its clean-up and concentration to be analyzed in the corresponding chromatographic system.

## Experimental

### Equipment

LC-DAD Agilent 1100 with:  
DAD G1315B operating at 313 nm for Coumaphos, 289 nm for Amitraz.  
Automatic Injector Agilent G1367B  
Column: Phenomenex Luna C18 100A 250 mm x 4,6 mm x 5µ

HRGC-uECD Agilent 6890  
Automatic Injector Agilent 7683B  
Electronic Pressure Control  
Column: Zebron ZB-5 60 m x 0,25 mm x 0,25 µm

HRGC-LRMS  
GC Agilent 6890N  
Mass Selective Detector Agilent 5973 Inert  
Automatic Injector Agilent 7683B  
Electronic Pressure Control  
Column: Agilent HP-5MS 30 m x 0,25 mm x 0,25 µm

### Chromatography Conditions

HPLC-DAD  
Mobile Phase: acetonitrile:water pH=9 (70:30).  
Flow: 1mL/min  
Oven Temperature: 25°C

### HRGC

Carrier Gas: Helium  
Flow: 1 ml/min  
Oven Programming Temperatures: T<sub>i</sub>=40°C R<sub>1</sub>=50°C/min up to 180°C R<sub>2</sub>=10°C/min up to 230°C R<sub>3</sub>= 5°C/min up to 280°C during 35 min.  
Injector Temperature: 240°C Mode Splitless  
uECD: Detector Temperature = 350°C  
MS: Source Temperature = 230°C, Quad Temperature = 150°C, Electron Impact 70eV, Interface Temperature = 280°C

### Sample Preparation

The sample is extracted with hexane:isopropanol:ammonium hydroxide 60:30:1. The organic layer is washed with 0,28 % Ammonium Hydroxide and concentrated to dryness. The residue is reconstituted with 1 mL acetonitrile. Due to matrix effects the calibration curves are prepared in blank matrix for the pesticides analyzed by GC (Coumaphos and Fluvalinate). For Amitraz, analyzed by HPLC the calibration curve is prepared in solvent.

## Validation

### Accuracy

#### Trueness

We use as a measure of trueness the recovery. The recovery and trueness were determined on spiked blank samples at different levels. One level of spike is the MRL, and the others are above and below it. The mean recoveries are shown in table.

#### Precision

For repeatability (RSD<sub>r</sub>) we analyzed in the same day a minimum of 5 replicates with the same analyst. For intermediate reproducibility (iRSD<sub>r</sub>) we repeated the analysis during at least 3 different days. Acceptance criteria RSD < 20 for levels above and equal the LOQ. The repeatability and intermediate reproducibility are shown in table.

		AMITRAZ		FLUVALINATE		COUMAPHOS	
Repeatability	LEVEL (ug/Kg)	10	200	10	50	10	80
	RSD <sub>r</sub> (%) - n=5	21,8	8,8-20,1	9,8-11,0	5,8	7,8	9,6
Intermediate reproducibility	Mean Recovery (%)	88,3		97,0		85,9	
	IRSD <sub>r</sub> (%) - n=3	21,6		11,2		12,1	

### Acknowledgments

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### Detection limit (LOD) and Quantitation Limit (LOQ)

To measure LOD and LOQ we spike a minimum of 5 replicates of blank samples. The LOD and LOQ are 3 and 10 times the signal-to-noise ratio (S/N).

**LOD: 3ug/Kg**  
**LOQ: 10ug/Kg**

### Linearity / Calibration Curve

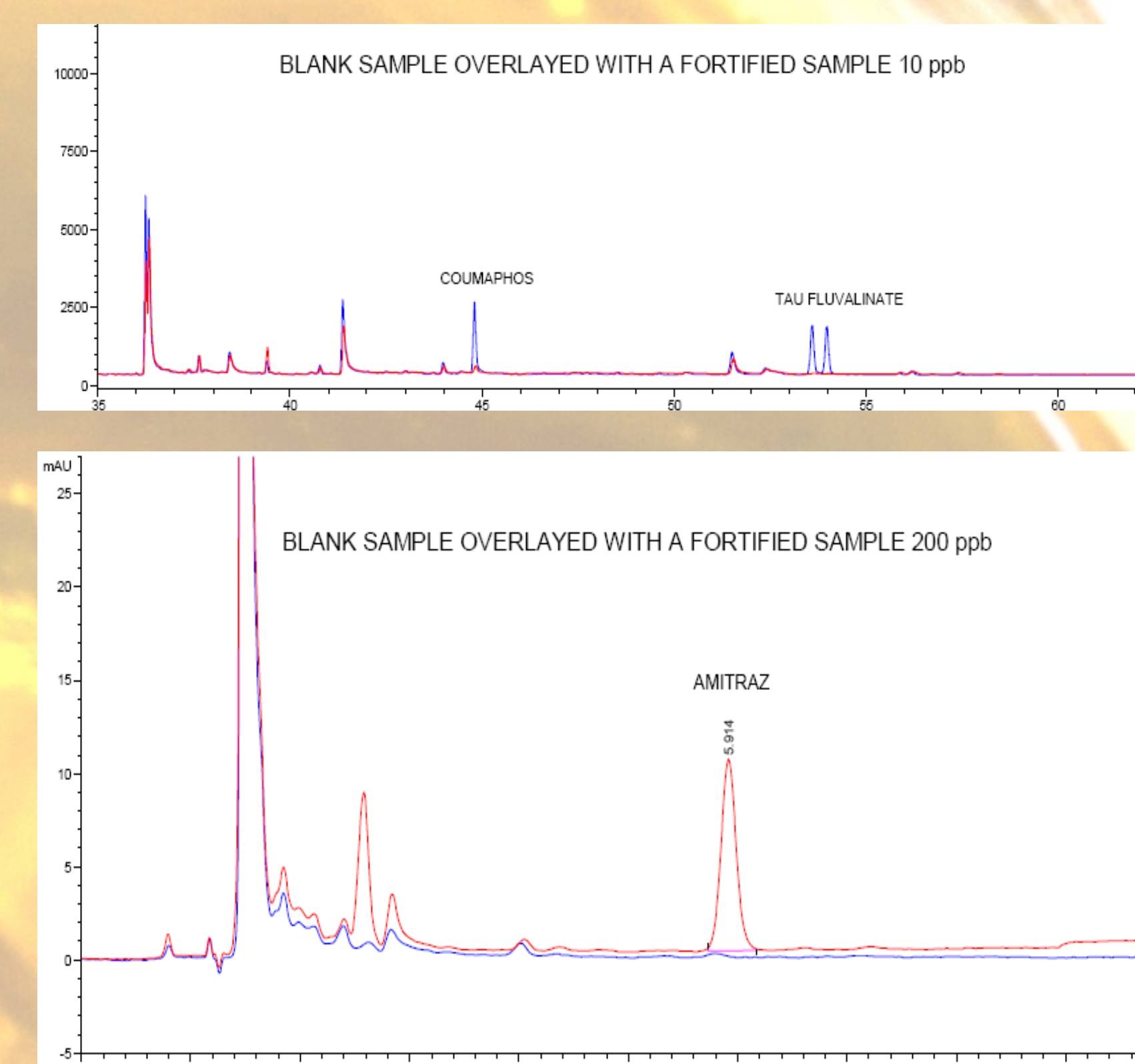
To avoid matrix effects, matrix-matched calibration standard curves were selected to quantify the pesticides analyzed by GC, Coumaphos and Fluvalinate. Blank honey samples were spiked with variable amounts of pesticide of interest and alfa-endosulfan is also added as internal standard.

	AMITRAZ		COUMAPHOS		FLUVALINATE
	HPLC	HPLC*	HPLC	GC	
RANGE (ug/Kg) in sample	10 – 200		10 – 260	10 – 200	10 – 200
r <sup>2</sup>	0,999	0,999	0,999	0,998	0,999
n	5	5	9	6	5

\* The data for this calibration curve is made with standard in solvent.

### Selectivity

Blank samples analyzed in the batches show no interferences. For routine analysis HPLC-DAD and HRGC-uECD are used. The confirmation of positive results are confirmed by mass spectrum and UV spectrum.



### Uncertainty 25%

Expanded uncertainty, calculated using sum of squares of type A and B components of uncertainty with a probability of 95% k=2.

## Conclusions

The analyzes presented here are sensitive, accurate and useful for routine analysis.

When we compare the results of Amitraz using matrix matched calibration and standard in solvent calibration, no significant differences are shown,

## Bibliography

- 1- Journal of Chromatography A, 954 (2002) 173-180
- 2- SANCO/10684/2009. Method Validation and Quality Control Procedures for Pesticides Residues Analysis in Food and Feed.