



Quantification of Pesticides in Rat Adipose Tissue: Development and Validation of a Simple, Fast and Sensitive Method.



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Introduction

The presence of pesticides in environmental and biological materials have been known for decades, but studies regarding their low concentration potential carcinogenic and endocrine disruption effects are only beginning. Therefore, the development of quantitative analytical methods for pesticides in complex biological samples is important not only to estimate pesticide accumulation but also to evaluate possible tissue damages. The aim of this work was to develop and validate a simple, fast and sensitive method for the pesticides dicofol, dieldrin, endosulfan and permethrin in rat adipose tissue. These specific molecules have been selected because they are the most frequently found in fruit and vegetables consumed in Brazil, according a survey performed by the Brazilian National Health Surveillance Agency (Anvisa - Agência Nacional de Vigilância Sanitária) and because they have been recently found to affect the endocrine system.

Experimental

1. Extraction

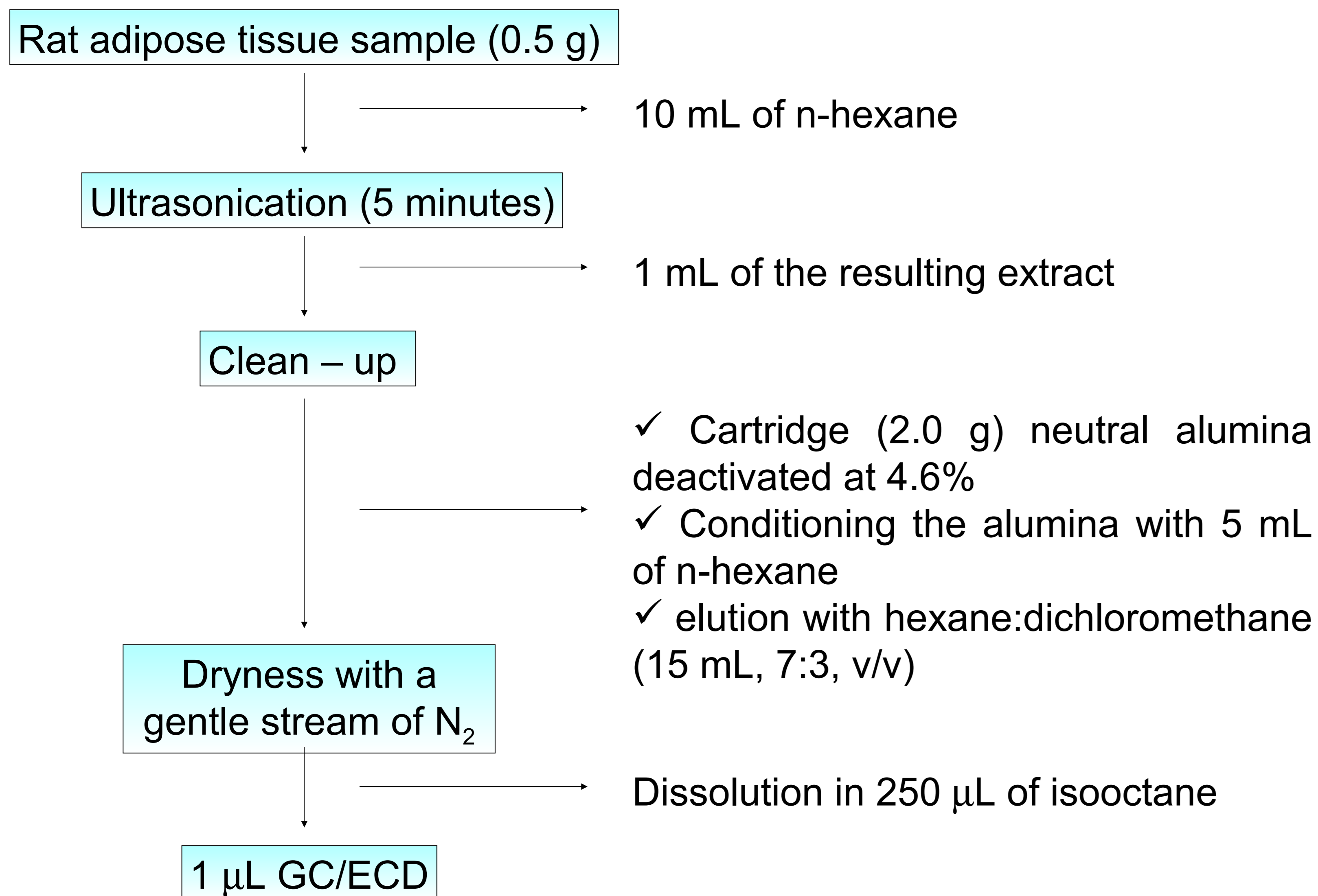


Figure 1: Extraction method for determination of pesticides residues in rat adipose tissue

2. Analysis by GC/ECD

- Equipment: Varian 3800 Gas Chromatograph equipped with ⁶³Ni Electron Capture Detector (GC/ECD)
- Capillary Column: V-5MS (30 m × 0.25 mm i.d., film thickness 0.25 µm)
- Oven Temperature: 200 °C (4 min) → 5 °C min⁻¹ → 280 °C (6 min)
- Volume Injection: 1 µL (manual injector, splitless mode)
- Injector Temperature: 250°C
- Detector Temperature: 300°C
- Nitrogen (purity 99.999%) was used as carrier and makeup gas at 33,0 mL min⁻¹

3. Validation of Method

- Linearity range***: 5 – 120 ng g⁻¹ for Endosulfan and Dieldrin
80 – 1200 ng g⁻¹ for Dicofol and Permethrin
*analytical curve in control sample extract (evaluation of matrix effect)
- Accuracy**: recovery at levels of control sample fortification
0.05 e 0.5 mg kg⁻¹ for Endosulfan and Dieldrin
0.5 e 5 mg kg⁻¹ for Dicofol and Permethrin
- Precision (repeatability)**: evaluated by coefficient of variation (CV)
- Selectivity**: analysis of the control samples (pesticides free adipose tissue samples)
- Method quantification limits (LOQ)**: calculated according to the THIER and ZEUMER¹ criteria (manual of pesticide residue analysis)

Results

Selective method

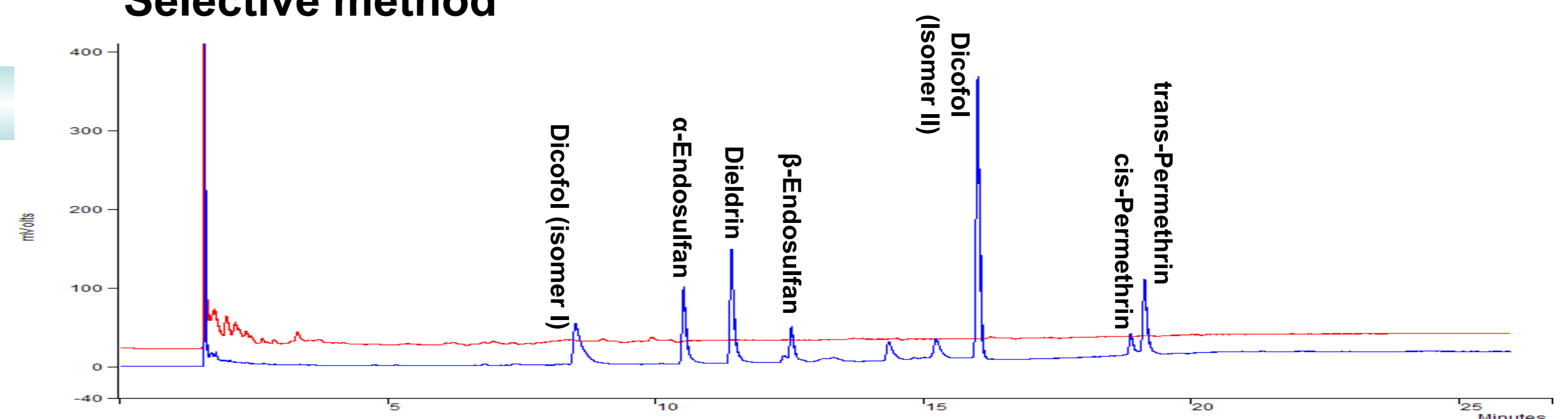


Figure 2: Chromatograms of sample control (red) x pesticides standard solution (blue)

Table 1: Recoveries of pesticides from fortified rat adipose tissue samples

Pesticides	Fortification Level (mg kg ⁻¹)	Recovery (%)	CV* (%)	Method Quantification Limits (mg kg ⁻¹)
Dicofol	0.5	87	18	0.1
	5.0	88	3	
α-Endosulfan	0.05	92	12	0.01
	0.5	111	6	
Dieldrin	0.05	94	10	0.01
	0.5	116	4	
β-Endosulfan	0.05	75	7	0.01
	0.5	106	7	
cis-Permethrin	0.5	86	8	0.1
	5.0	119	14	
trans-Permethrin	0.5	82	19	0.1
	5.0	101	12	

*Coefficient of variation (CV), n = 3

Conclusion

The method developed is more simple and faster than existing methods², shows good validation parameters and allows simultaneous determination of the pesticides in studies with a large number of samples and complex matrixes such as rat adipose tissue.

[1] THIER, H.P.; ZEUMER, H., *Manual of pesticide residue analysis*, 1987.

[2] KUVAREGA, A. T.; TARU, O., *Environmental Monitoring and Assessment*, 2007, 125, 333 – 345.

ACKNOWLEDGMENTS:

