Comparison Between the Efficacy of Two Cleanup Methods for the ^{1}H NMR Analysis of Food Samples Contaminated with Cypermethrin

M. M. Silva, J. D. Figueroa-Villar, A. P. Aguiar
Departamento de Química, Instituto Militar de Engenharia,
Praça General Tibúrcio 80, 22290-270 Rio de Janeiro, Brazil

C. A. S. Riehl*
Departamento de Química Analítica, Instituto de Química - UFRJ
Av. Trompowisky s/n, 21945-970 Rio de Janeiro, Brazil
riehl@iq.ufrj.br

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Abstract: *This work aimed to study the use of ^{1}H NMR for the identification of cypermethrin in cooked foods. ^{1}H NMR is not commonly used in these cases, because food samples ready for consumption have complex substances, mainly lipids, which usually interfere with the identification of cypermethrin. Thus, we drew a comparison between the most applied method for the treatment of those samples and an alternative route that made possible the use of ^{1}H NMR in the identification of cypermethrin in a matrix consisting of rice, bean, and chicken, which allows the Forensic work for such cases.*

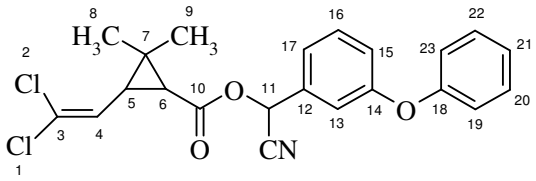
Resumo: *Este trabalho teve por objetivo avaliar o emprego da RMN^{1}H em análises de alimentos cozidos para a identificação de cipermetrina. A RMN^{1}H é pouco utilizada nestes casos, pois amostras de alimentos processados para o consumo, apresentam substâncias complexas, principalmente triglicerídeos, que geralmente interferem na identificação da cipermetrina. Portanto, neste trabalho foi realizada uma comparação entre o método mais aplicado para o tratamento dessas amostras e uma rota alternativa, que possibilitou o uso da RMN^{1}H na identificação da cipermetrina em matriz composta por arroz, feijão e frango, viabilizando o trabalho Forense para esses casos.*

Introduction

Cypermethrin (**1**) is a synthetic pyrethroid frequently used as a pesticide. The synthetic pyrethroid insecticides have their origin in the important botanical insecticide pyrethrum, an extract obtained from the flowers of *Chrysanthemum cinerariaefolium*. The six natural esters of pyrethrum are pyrethrins, jasmolins, and cinerins (**2** to **7**), which are effective against a wide range of household and public health insects.[1] People exposed to pyrethroids, do not only include workers in the chemical industry (production, filling, formulation), farmers and pest control operators but also consumers. Nowadays, pyrethrum is formulated as aerosol or spray with synergists (e.g. pireronyl butoxide) for use as a rapid knock-down or flushing agent in the industry and in the home. Suicides and accidents due to poisoning with pesticides are occasionally encountered in forensic practice.

Pyrethroides are very lipophilic components with neurotoxic properties, interacting with the sodium channel both in insects and mammals. In mammals, they are rapidly metabolized by cleavage of the central ester linkage, leading to non-toxic metabolites. Consequently, the toxicity of pyrethroids for mammals is classed as low. However, recent investigations showed interindividual differences in pyrethroid metabolism, concerning the activity of carboxylesterases responsible for the detoxification.[2]

The pyrethroids identification in cooked foods composed mainly of rice, bean and chicken is very important in forensic practice. These foods are consumed more often by Brazilians. Concerning the ^{1}H NMR analysis of food samples one of the worst problems is the elimination of the fraction composed of lipids from such samples.



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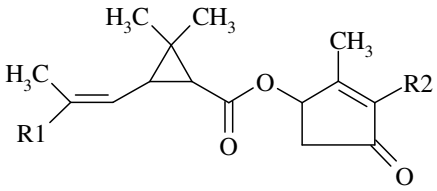

Rethrin	R1	R2
2 Pyrethrin I	CH_3	$CH_2CH=CHCH=CH_2$
3 Jasmolin I	CH_3	$CH_2CH=CHCH_2CH_3$
4 Cinerin I	CH_3	$CH_2CH=CHCH_3$
5Pyrethrin II	$COOCH_3$	$CH_2CH=CHCH=CH_2$
6 Jasmolin II	$COOCH_3$	$CH_2CH=CHCH_2CH_3$
7 Cinerin II	$COOCH_3$	$CH_2CH=CHCH_3$

Experimental

In this work, standard cypermethrin (92.5%) obtained from Johnson was used. NMR measurements were carried out on a Bruker DRX-200 (200 MHz) NMR spectrometer.

Florisil, 60-100 mesh purchased from Merck, was used after heating in an oven at 250°C for 5 h, cooling and addition of 5% water.

Fortification of the Matrix with Cypermethrin

The composition of the matrix was rice, bean, and chicken in a ratio of 1:1:0.5. The chicken was unraveled and mixed together with the bean and the rice. Subsequently, 20 mg of cypermethrin were added to 40 g of matrix in a beaker and then mixed together.

Extraction of Cypermethrin from the Matrix

Dichloromethane (10 ml) was added, and the solution was stirred for 2 minutes. Afterwards, the dichloromethane was separated from material to be extracted. The other two extractions were accomplished with 5 ml of dichloromethane under the same conditions, except for stirring time (1 minute). At the end of each extraction process, the extracts were placed in a beaker for 2 hours for solvent evaporation.

Preparation of the Florisil Adsorption Column

Florisil (10 g) mixed with hexane was transferred slowly into a glass column, and 1cm layer of anhydrous granular sodium sulfate was added. Hexane level reached the top of the Florisil column.

Preparation of the Florisil Partition Column

Florisil (10 g) mixed with acetonitrile equilibrated with hexane was transferred slowly into the glass column and 1cm layer of anhydrous granular sodium sulfate was added. The excess acetonitrile that was not adsorbed on Florisil was washed out with 20 ml hexane equilibrated with acetonitrile. In order for both solvents to be in equilibrium, they were placed into a flask and then mixed.

Cleanup (applied for partition column and adsorption column)

The extract was transferred into the 8 g of Florisil column prepared previously. The solution passed through the column until the liquid level reached the top of the column. The beaker was rinsed three times with 2 ml of hexane, and the washes were transferred to the column, which was washed with 20 ml of hexane to remove lipids. The pyrethroid was eluted with 50 ml ethyl ether-hexane (3+2), and the eluate was collected in a beaker. The eluate was evaporated on a rotary evaporator at 40 ^{o}C.

Results and Discussion

Figure 1.A shows the spectrum of the sample derived from the matrix without cypermethrin. The spectrum A is similar to the spectrum of lipids present in olive, sunflower and soybean.[4] The extraction of this matrix was performed in the same way as that for the extraction of cypermethrin from the matrix that contained the pyrethroid. Figure 1.B shows the ^{1}H NMR of the extracted material derived from the matrix that contained cypermethrin. Before NMR analysis, this material was washed from the adsorption column. These two spectra were very similar. Probably, the lipids were mostly washed out together with cypermethrin or the pyrethroid was adsorbed in the Florisil column. These results do not point to a satisfactory use of ^{1}H NMR for the identification of cypermethrin in foods.

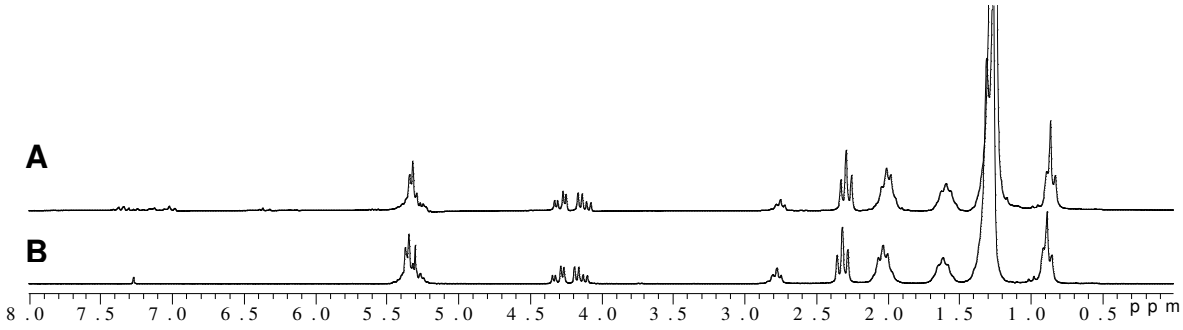

Figure 1. A) ^{1}H NMR spectrum of the matrix with cypermethrin after using the adsorption column; B) ^{1}H NMR spectrum of the matrix without cypermetrhin.

Other cleanup methods were applied (Figure 2). The result of the partition column was shownin spectrum B. The spectrum of cypermethrin was acquired (Figure 2B).

These two spectra are quite similar. The cleanup showed to be efficient, because during the first elution with hexane the lipids were washed out of the column while the pyrethroids were adsorbed.

During the second elution with a mixture of solvents with more polar characteristics, cypermethrin was then recovered.

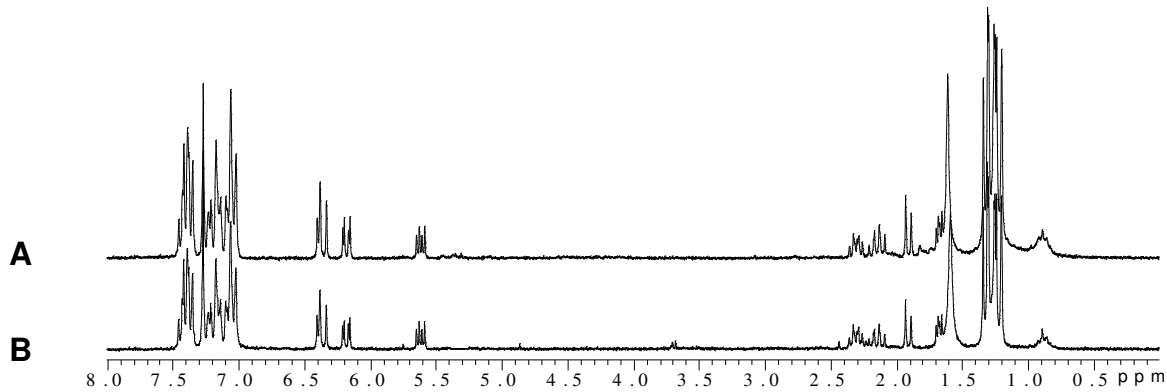

Figure 2. A) ^{1}H NMR spectrum of the matrix with cypermethrin after using the partition column; B) ^{1}H NMR spectrum of cypermetrhin.

Table 1. Peak assignments for ^{1}**H** NMR spectra of cypermethrin

Hydrogen Atomic Number*	Chemical shift (ppm)	Multiplicity
4 (isomers I and II)	6,15-6,21	Double doublet
4 (isomers III and IV)	5,59-5,65	Double doublet
5 (isomers I and II)	2,09--2,23	Multiplet
5 (isomers III and IV)	2,23-2,36	Multiplet
6 (isomers I and II)	1,89 e 1,93	Doublet
6 (isomers III and IV)	1,66-1,70	Double doublet
8 and 9	1,20-1,34	Multiplet
11(isomers I and II)	6,33	Singlet
11(isomers III and IV)	6,38 e 6,40	Doublet
13,15,16, 17 and 19-23	7,02-7,46	Multiplet
Unknown	1,59	Broad singlet

Table 1 shows the peak assignments for ^{1}H NMR of cypermethrin, in agreement with the results reported by Edwards. There are three chiral centers in cypermethrin, and, therefore, four diastereoisomers, which renders the interpretation of such a spectrum ambiguous. The configuration of the cyclopropyl ring has a marked effect on the toxicity of cypermethrin, with 1R-cis isomers being the most toxic. The benzylic carbon atom should subtend the α-cyano group in the (S)-configuration for maximum potency.[3]

Conclusion

This work demonstrated that the identification of cypermethrin both in cooked and ready for consumption foods can be made by ^{1}H NMR, as long as appropriate cleanup methods are used.

References

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