BENAKI PHYTOPATHOLOGICAL INSTITUTE

Validation of an HPLC analytical method for the determination of natural pyrethrins in a new microemulsion

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INTRODUCTION

Natural pyrethrins were among the earliest pesticides discovered by agriculturists. Pyrethrins are extracted from the powdered flower-heads of *Chrysanthemum cinerariaefolium*, and have been used as insecticides for many years. The term "pyrethrum" refers to the plant, the flower or the crude, concentrated or refined extracts, and the term "pyrethrin" is reserved for describing the active ingredients of pyrethrum. The natural pyrethrins are non-persistent insecticides with a low toxicity to mammals.

Changes in agricultural technology have been a major factor shaping modern agriculture. Among the latest line of technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production. The development of nanodevices and nanomaterials, could open up novel applications in plant biotechnology and agriculture. In that case natural pyrethrins were nano-formulated in water-in-oil (w/o) microemulsions based on safe, biocompaticle materials such as lemon oil terpenes as dispersant, polysorbates as stabilizers and mixtures of water with glycerol as the dispersed aqueous phase.

The analysis of natural pyrethrins has been of interest for many areas, especially in the insecticidal industry where pyrethrum extracts are used in formulations. Six insecticidally active pyrethrin esters namely, pyrethrin I, jasmolin I, cinerin I, pyrethrin II, jasmolin II and cinerin II, have been isolated from the pyrethrum extracts. Due to their thermal instability, HPLC methods are preferred over GC methods for analyzing pyrethrins and related compounds. Reversed phase high performance liquid chromatography was used in the past due to the wider separation ability and lower solvent cost. Nonpolar components, such as pyrethrins and pyrethroids, are strongly retained on the RP columns, while very polar compounds are only slightly retained.

The objective of the present project was the development of a new technology of micromaterials such as microemulsions and their use in the processing and packaging of organic plant protection products in order to enhance the biocidal action while reducing the environmental charges. The new microemulsion was set under chemical control and insecticidal activity. The first included the assessment of the physicochemical properties under storage stability tests in low and high temperatures and the validation of an analytical method for the determination of the content of the six components. The insecticidal activity included the evaluation of the effect of the natural pyrethrin microdispersions in laboratory and semi-field bioassays upon a target-insect pest, the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) in eggplant.

An isocratic Reversed Phase HPLC method was developed for the determination of the six pyrethrin components in two different batches of samples and validated for specificity, linearity, precision, and accuracy.

EXPERIMENTAL

Materials and methods

Two different batches of samples named System II-a (SII-a) and System II-b (SII-b), which contained lemon oil terpenes, tween 20, H2O/Glycerol and pyrethrins at a concentration of 3.5% w/v and a blank were provided to the Laboratory, in order to evaluate the stability of the new product and the repeatability of the production process. The blank formulation was used for the determination of recovery at three spiked levels at 1000μg/ml, 500μg/ml and 250 μg/ml.

The analytical standard used for the quantification of pyrethrins in the new nanoemulsion was purchased from Dr Ehrenstorfer (purity for pyrethrins: 43%). The stock solution was prepared in acetonitrile (ACN) at 1000μg/ml. A series of dilutions of the stock solution (from 10μg/ml to 1000μg/ml) were prepared also in acetonitrile, to study the linearity.

The preparation of samples included extraction with ACN, sonication for 60 minutes and filtration through a PTFE filter before RP-HPLC analysis.

Stability of liquid formulations at 0 ºC

The stability test at low temperatures was held, in order to record whether any solid or oily matter is present in the new nanoemulsion according to the official CIPAC method (MT 39).

Accelerated Storage Procedure

The objective of this procedure was to accelerate the ageing of the new nanoemulsion by heating and was done at 54ºC for 14 days according to the official CIPAC method (MT 46) . After this period of time, the samples were analyzed by the developed RP-HPLC-UV method.

Chromatographic Instrument and Conditions

A new isocratic RP-HPLC was developed for the determination of the pyrethrins in the microemulsion. A Shimadzu LC-20AB liquid chromatograph was used, equipped with on-line degasser, heated column compartment and automatic sampler and injector was used. Analysis was performed on a reversed phase HPLC-column, Luna C18, (250 x 460mm, i.d. 5 μm). The flow rate was set at 1.4ml/min. The temperature of the column compartment was maintained at 40ºC and the injection volume was 10μl. The six pyrethrins were absorbed at the wavelength of 225nm. The analysis time was 40 min. In order to achieve good separation, a two component mobile phase with isocratic elution, consisting of acetonitrile and water was used in a ratio of 65/35 respectively as shown in figures 2-5.

RESULTS AND DISCUSSION

The new RP-HPLC method was validated in accordance with CIPAC guidelines (Collaborative International Pesticide Analytical Council, 2003). Chromatographic method validation, consisting of method linearity, accuracy, specificity, and precision, was undertaken in order to demonstrate the suitability of the analytical method for the determination of natural pyrethrins. The results presented as follows showed the repeatability of the production process (Fig.1)

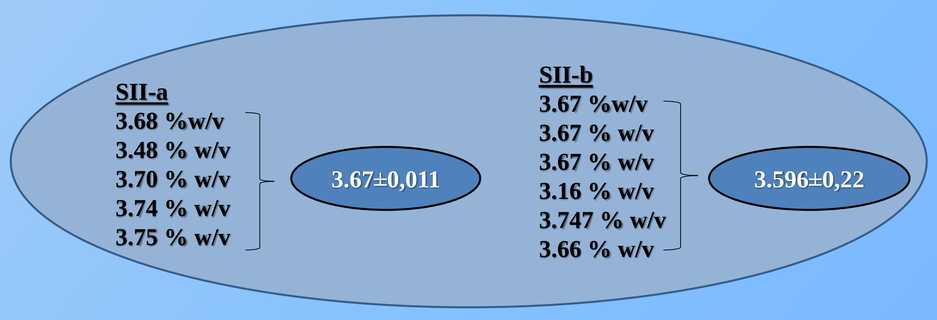

Figure 1: Concentration of pyrethrins in two different batches

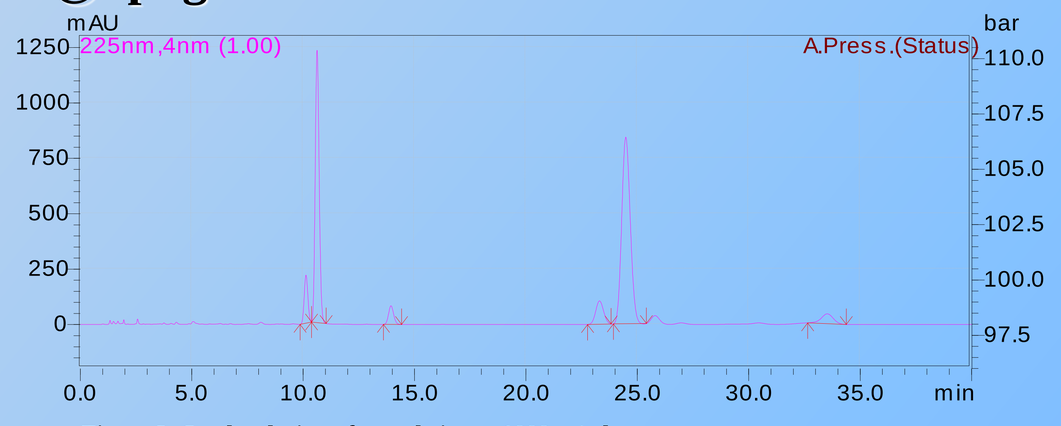

Figure 2: Stock solution of pyrethrins at 1000μg/ml

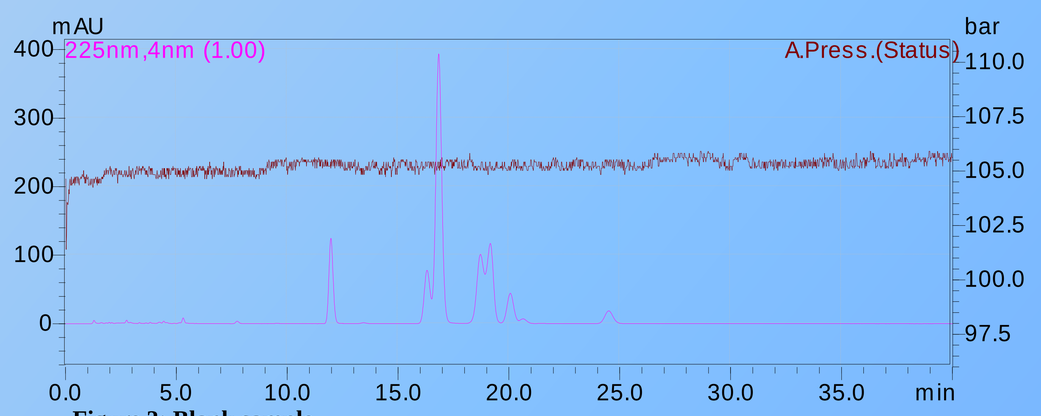

Figure 3: Blank sample

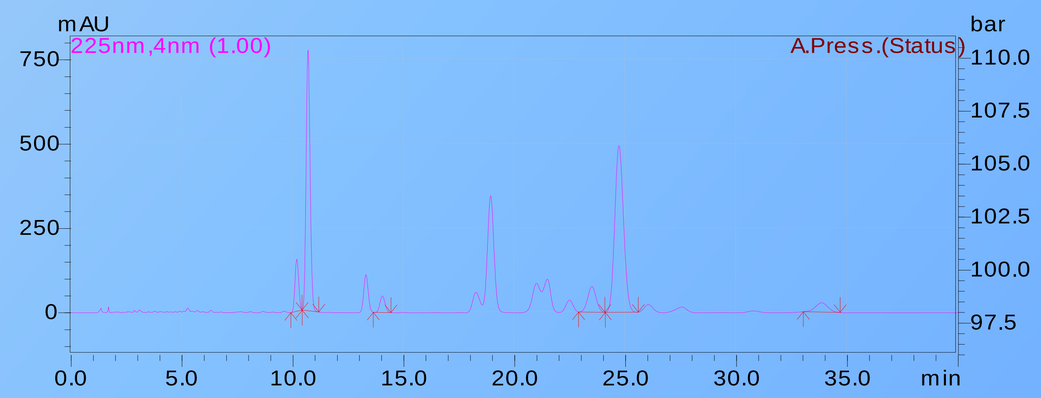

Figure 4 : Chromatographic Profile of System II-a

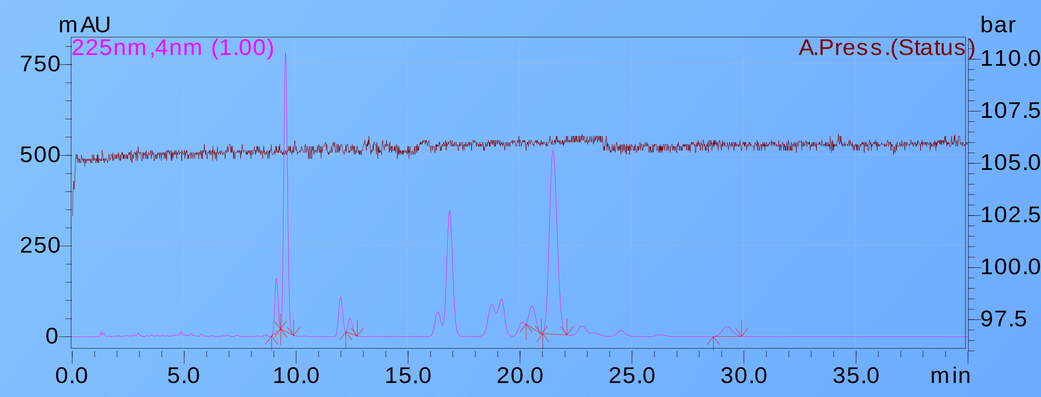

Figure 5: Chromatographic profile of System II-b

CONCLUSIONS

It was proved that the new product is stable during storage stability tests, as the concentration of the pyrethrins was reduced less than 3% and also the production process was reproducible. The insecticidal activity against the cotton aphid *A. gossypii* showed that the new natural pyrethrin microemulsion had increased efficacy compared to the natural pyrethrin emulsion. In addition, there was no effect on the aphid's natural enemy *Coccinella septempunctata* (Coleoptera: Coccinellidae), which is desirable for the control of this pest.

Acknowledgements

This study has been supported by the program 'Development of a new microemulsion as new materials for managing the plant protection products and for the reduction of environmental contamination', which is funded by the General Secretariat of Research and Technology.

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