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Higher Nature Limited
Mrs. Celia Wright
Burwash Common
Eat Sussex
TN 19 7LX

PHYTOCHEMICAL PROFILE OF HERB SAMPLE

Dear Celia,

Please find details enclosed consisting of a report and experimental methods which were undertaken to answer your questions. However for your convenience I have sumarised the findings:

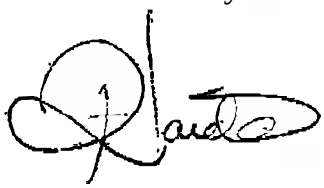
1) The analytical data gives the apparent Triclosan content of the sample of Citricidal as received was 27 p.p.m., as determined by HPLC.

2) Fluorescence spectroscopy and UV spectroscopy indicated that the sample Citricidal does not contain neither benzalkonium chloride or benzethonium chloride.

The enclosed report contains the full details in support of the above statements.

We look forward to hearing from you soon.

Yours sincerely



Dr John A Wilkinson
Senior Lecturer in Phytochemistry and Pharmacognosy

1. INTRODUCTION

This report contains details of consultancy work undertaken by Dr. John Wilkinson, on behalf of MUSL for the client „Higher Nature Ltd“, Burwash Common, East Sussex.

Higher Nature stated:

We market Citricidal, a grapefruit seed extract in Europe and are concerned to have tested in the UK.

What we want to know is:
1) Has the product been contaminated with Triclosan?

2) Does this product contain added benzethonium chloride or benzalkonium chloride?

A sample of Citricidal (2x 100ml) was sent to Dr Wilkinson for analysis.

2. INVESTIGATION

Suspected content: Triclosan

The determination of Triclosan was carried out routinely by an HPLC method; this was applied in its usual form, but it was found that the peak obtained at the retention time expected for Triclosan was interfered with by peaks due to other constituents.

The mobile phase composition was altered to attempt to remove this interterence, and finally conditions were derived which allowed the measurement of the peak at the Triclosan retention time with no problems. The retention time was actually slightly different from that obtained with pure solution standards, but this was not uncommon, especially in solutions derived from natural products, where viscosity, surface tension and ionic strength may all differ markedly from pure solutions. The peak obtained was clean and well shaped, and the addition of an equivalent amount of Triclosan yielded an increase in the peak height with no obvious peak distortion or impairment of its shape, so that assuming that the reasons for suspecting the presence of Triclosan are valid, the observed peak is most likely due to this compound.

However, in a natural product, with its plethora of possible constituents, a single , chromatographic peak obtained under a single set of chromatographic conditions is not an absolute guarantee of identity. It would be necessary to use either a selective identification technique such as HPLC/mass spectrometry, or to devise at least one other set of chromatographic conditions where good chromatograms were obtained and still showing identity of retention times if the confidence is to be improved.

Besult: The apparent Triclosan content of the sample of Citricidal as received was 27 p.p.m., as determined by HPLC.

Suspected content: Benzalkonium Chloride / Benzethonium Chloride

The method of analysis given below was found to be reliable down to 0.3 p.p.m. of both the above quaternary compounds.

Preparation of samples for spectroscopy

To enable an analysis of the two quaternary compounds the following method was developed which was found to be reliable and efficient with no interferences.

Two aliquots of the sample were taken, each being 100 ml, and labelled „A“ and „B“. To sample “A“ was added diethyl ether (150 ml) as the extractant. The extraction was carried out in a magnetically stirred vessel with the contents of the vessel heated to 20° C. Further additions of diethyl ether kept the volume to a convenient size. The ethereal extract was separated. The extraction procedure was repeated again using diethyl ether (150 ml.). The combined ethereal extracts were evaporated in vacuo at 20° C to give a pale amber oily film - residue „A“ , which was subsequently subjected to two different methods of spectrometric analysis.

To sample „B“ was added petroleum ether (150 mI.) as the extractant. The extraction was carried out in a magnetical1y stirred vessel with the contents of the vessel heated to 20° C. Further additions of petroleum ether kept the volume to a convenient size. The petroleum extract was separated. The extraction procedure was repeated again using petroleum ether (150 ml). The combined ethereaI extracts were evaporated in vacuo at 20° C to give a pale amber oily film - residue „B“.

Fluorescence spectroscopy

Standard samples of benzalkonium chloride and benzethonium chloride were found to be sensitive to fluorescence spectroscopy at concentrations as low as 0.5 p.p.m. using a Perkin Elmer 204 Fluorescence Spectrophotometer. No corresponding fluorescence was found for suitably prepared samples obtained from residues „A" or „B". It was suspected that the presence of Triclosan may have interfered with the observations, however on testing this hypothesis there was found to be no interference.

Result: *Fluorescence spectroscopy indicated that both residues „A" and „B" were free from the suspected quatemary compounds, implying that the sample of Citricidal as received was free of benzalkonium chloride and benzethonium chloride.*

Ultraviolet spectroscopy

Standard samples of Triclosan, benzalkonium chloride and benzethonium chloride were scanned on a dual beam Pye Unicam SP8000 U/V Spectrophotometer, all of which compounds gave good clean absorption spectra. This method of U/V analysis was found to be reliable down to 0.3 p.p.m. for both the above quaternary compounds.

Result: *Samples prepared from residues „A" and „B" which were taken up in demineralised water and scanned by U/V, only gave absorption indicative of the presence of Triclosan, but no indication of the presence of either benzalkonium chloride and benzethonium chloride in the sample of Citricidal as received.*

Further analytical checks on procedure

A sample of Citricidal as received was spiked with benzalkonium chloride and benzetbonium chloride and extracted by the methods given above. The quaternary compounds could be detected in the extracts by both fluorescence spectroscopy and U/V spectroscopy.

In order to reduce the possibilities of interference by other naturally occurring components, residue „B“ was subjected to flash chromatography with gamma aluminium oxide as the stationary phase and petroleum ether/ethyl acetate as the mobile phase. Twenty samples of eluant (1 ml) were collected. Evaporation of the final six samples in vacuo at 20°C gave pale amber oils, which were taken up in demineralised water and scanned by U/V as before. None of the U/V spectra of the six samples indicated the presence of the suspected quaternary compounds.

Result: *Both fluorescence spectroscopy and U/V spectroscopy indicated that the sample of Citricidal received contained neitber benzalkonium chloride or benzethonium chloride. These spectroscopic methods did however confirm the presence of Triclosan. Quaternary compounds in Cidricidal resulting from the process of its extraction from grapefruit.*

Despite the discovery that the suspected quaternary compounds were not found in the sample of Cidricidal as received, a literature search was still carried out on this topic. A search of both Chemical Abstracts and Biological Abstracts revealed no reports to date of quaternary compounds being found in citrus extracts.

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