Document 89/8

Solid Phase Extraction of Methoprene Residue from Tobacco with On-Line Elution and Subsequent HPLC Analysis*

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ABSTRACT

The analysis of hexane extracts of tobacco containing methoprene residues is performed using two solid-phase extraction cartridges in tandem, followed by automated, on-line elution and reversed-phase HPLC. Replicate analyses (n=10) of a flue-cured tobacco sample having a mean methoprene residue of 1.89 ppm gave a standard deviation of 0.05 and a coefficient of variation of 2.6%. A standard addition plot was linear in the range 0.5-11 ppm (r^2=0.999). No solvent evaporation steps are required and all glassware and plasticware items are disposable. Approximately 40 samples can be prepared for unattended HPLC analysis by one analyst in a normal work day.

INTRODUCTION

Inspiration for analytical work regarding methoprene residues can be attributed to the insect pest, *Lasioderma serricorne*, commonly known as the cigarette beetle. One very promising method for control of this insect involves application to tobacco of a synthetic insect growth regulator known as methoprene. Methoprene is the active principal of Kabat and Dianex which are manufactured by the Zoecon Corporation.

Insect growth regulators (IGRs) comprise a group of compounds classified by the United States Environmental Protection Agency as "biochemical pesticides". Methoprene has extremely low mammalian toxicity, is EPA-approved, and is sanctioned by the World Health Organization for use in potable water. The compound is effective because it mimics the biological activity of a structurally similar, naturally-occurring cecropia juvenile hormone (1).

Methoprene is 100% effective in the laboratory at a level of one ppm (2).

Methoprene (Figure 1) is totally synthetic and differs from the natural hormone in several structural details. Most notable in the synthetic material is the existence of a 2,4-dienoic ester chromophore that is not present in the natural product. This feature was added for enhancement of potency. Coincidentally, it also lends UV detectability due to its absorption of light at 259 nm. The Zoecon Corporation, manufacturers of methoprene, also provide upon request a secondary butyl ester variant referred to as MPM. MPM is a superb internal standard for methoprene monitoring since it responds very similarly in FID and UV detection systems. Its chromatographic behavior

is very close to that of methoprene, but the two compounds are readily baseline resolvable in both GC and HPLC systems.

Monitoring of methoprene residues is performed largely for the purpose of ensuring appropriate application rates and efficacy of control. Since 1976, various proprietary and published GC (3-5) and HPLC (6,7) methods have been employed for analysis of numerous animal and vegetable matrices. In 1983, an HPLC method was established in our laboratory (6) that consisted of a hexane extraction, semi-preparative normal-phase HPLC, followed by reversed-phase end analysis. This mode-switching HPLC approach was highly successful in that methoprene and MPM appeared as the major peaks in the resulting reversed-phase chromatogram. Although an improvement over some alternative methods, sample throughput by this procedure became grossly inadequate for present day requirements. Consequently, a new method was developed.

EXPERIMENTAL

Apparatus: A model 5000 HPLC instrument (Varian Associates, Inc.) equipped with UV-50 variable wavelength UV detector was used. Configured to this instrument was an Advanced Automated Sample Processor (AASP) and a Vista 402 data and instrument control system (both Varian); the instrument was configured to an in-house CALS (Beckman Instruments) data system as well. Solid phase extraction (SPE) cartridges were prepared on a manual AASP Prepstation that was modified to accommodate two cassettes using an adapter kit (Varian Cat. No. 03-906578). Centrex Microfilters (Schleicher & Schuell)

with 0.2-micron nylon membranes and 5-ml receiver tubes were used as extraction vessels/filtration devices. Jet Pipets (Cole-Parmer) were used for convenient dispensing of extractant and solvent.

Solid phase extraction procedure: Stock solutions of methoprene and MPM were prepared by dissolving 10 mg of these materials in hexane and diluting to 100 ml. The manufacturer's stated percentage purities of the gratuitous samples were used in computing the titers of the solutions; these were stored at -20° C when not in use. The extraction solution was prepared every few days by dilution of one ml of the MPM stock solution to 250 ml (0.4 µg/ml). Ground tobacco (0.500 g) weighed to the nearest mg in a glass weighing funnel (Fisher Scientific, Cat. No. RNG311010, 1 x 3.5 cm) is placed in the upper portion of a Centrex Microfilter. Four ml of MPM extraction solution is delivered to the microfilter using a Jet Pipet. The microfilter is tightly capped, agitated vigorously for 30 sec on a vortex mixer, then allowed to stand for 30 min prior to centrifugation for 10 min at 3000 rpm.

A silica (SI) AASP cassette is attached to an AASP Prepstation; an aminopropyl (NH_2) AASP cassette is then fitted to the SI cassette using a stainless steel adapter supplied with the adapter kit. A solvent reservoir cassette is positioned over the NH_2 cassette. One-half ml of centrifugate from the above treatment is delivered to the reservoir chamber positioned over the desired cartridge. The entire array (Figure 2) is kept in place by a positive pressure manifold which is sealed to the solvent reservoir by tightening two thumb screws. Sample solutions are forced through the

cartridges under 1-2 psig of nitrogen (until the miniscus of the slowest percolating sample just reaches the top of the NH_2 bonded phase bed). At this point the toggle valve controlling the flow of nitrogen is switched off and the pressure manifold is removed. Methoprene + MPM are eluted from the NH_2 cassette on to the SI cassette using 1 ml of 30% (v/v) methylene chloride in hexane delivered from a Jet Pipet and repetition of the elution process. Nitrogen is allowed to pass through the cassettes for approximately five minutes following the elution step to remove residual solvent. The SI cassette is then placed in the hopper of the AASP in preparation for automated HPLC analysis.

Chromatography: On-line HPLC is performed using a Bio-Sil ODS-5S column (Bio-Rad Laboratories, 250 x 4 mm) with detection at 275 nm. Separation of methoprene and MPM is performed isocratically with 15% water in methanol and a flow rate of 1.0 ml/min. However, a steep gradient to 100% methanol, returning to 85%, is performed subsequent to elution of MPM; flow rate is increased to a maximum of 2.0 ml/min during this period.

During normal operation the AASP is placed in remote mode. However, in preparation for each analysis session, it is necessary to enter the number of the first cartridge, the number of samples, run time (37 min) and valve reset time (0.7 min) through the AASP keypad.

Quantification of methoprene was performed by the Vista 402 using internal standard methodology and peak heights. However, raw data are frequently collected and stored by the backup CALS 1 computer system. Calibration samples are prepared by combining equal volumes of 0.8-ppm solutions of

methoprene and MPM, followed by deposition of 0.5 ml of the resulting solution directly onto silica cartridges and passage of nitrogen for 5 min.

RESULTS AND DISCUSSION

Method Validation: A typical chromatogram of a flue cured tobacco sample (3.2 ppm methoprene) is shown in Figure 3. The precision of the new method was established by replicate (n=10) measurements of a 1.89 ppm tobacco sample using both peak heights and peak areas on both data systems (precision was found to be independent of the particular data system); use of peak height slightly out-performed peak area. The Vista 402 system, which showed a sample standard deviation of 0.05 (2.6% CV) was selected for routine use and the standard addition experiments that followed (Figure 4).

Advantages of this approach are good sample throughput (approximately 40 samples/day for one analyst), not having to contend with solvent evaporation steps, and the fact that all glassware/plasticware items are disposable. Over 5000 analyses have been performed using this approach since its adoption in 1987. Shown in Figure 5 is a time series plot of a 54-sample set of burley tobaccos processed at a production facility in 1986. This chart shows an initial application rate that was close to the target of 2.5 ppm, but which drifted lower with continued operation of the plant. Noticeable also is one case of over-application.

ACKNOWLEDGMENT

Methoprene is used to control a variety of other insects in a variety of

stored grains. We have successfully applied this procedure to corn, wheat, oats, barley and milo and we thank Mr. Terry Pitts of the Gustafson Co. for supplying these samples.

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CAPTIONS FOR ILLUSTRATIONS

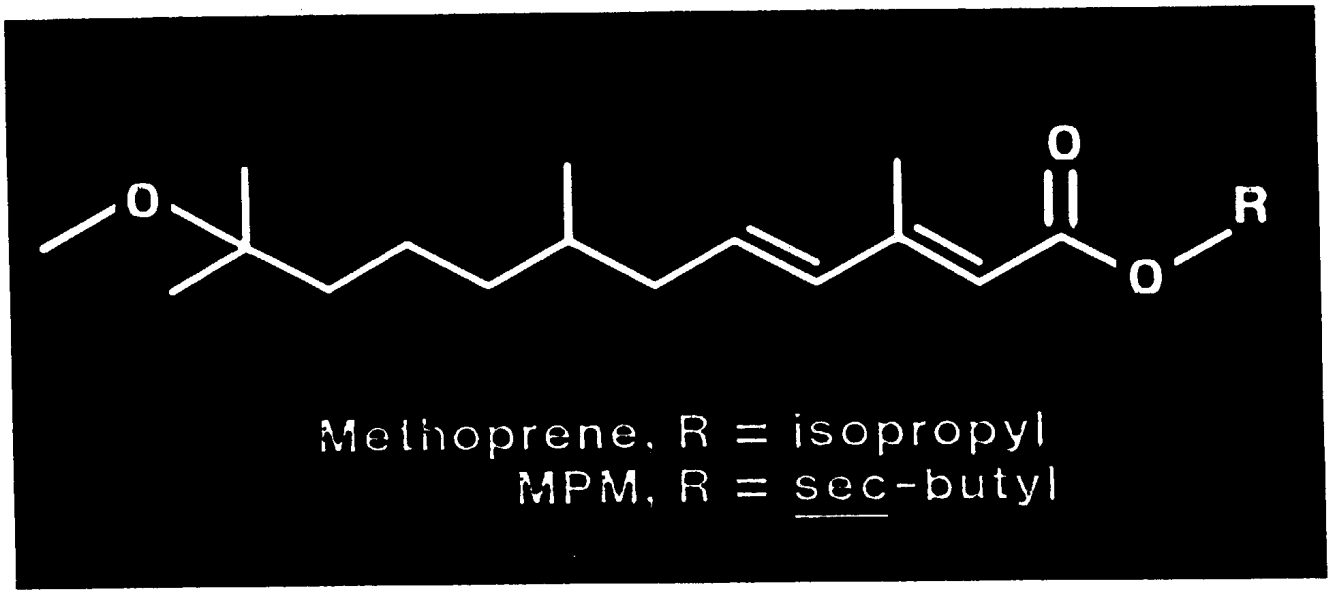
FIGURE 1: Structures of methoprene and MPM.

FIGURE 2: AASP Prepstation with cassettes in place.

FIGURE 3: Chromatogram of flue-cured tobacco extract following preliminary fractionation by SPE.

FIGURE 4: Standard addition plot of methoprene in flue-cured tobacco.

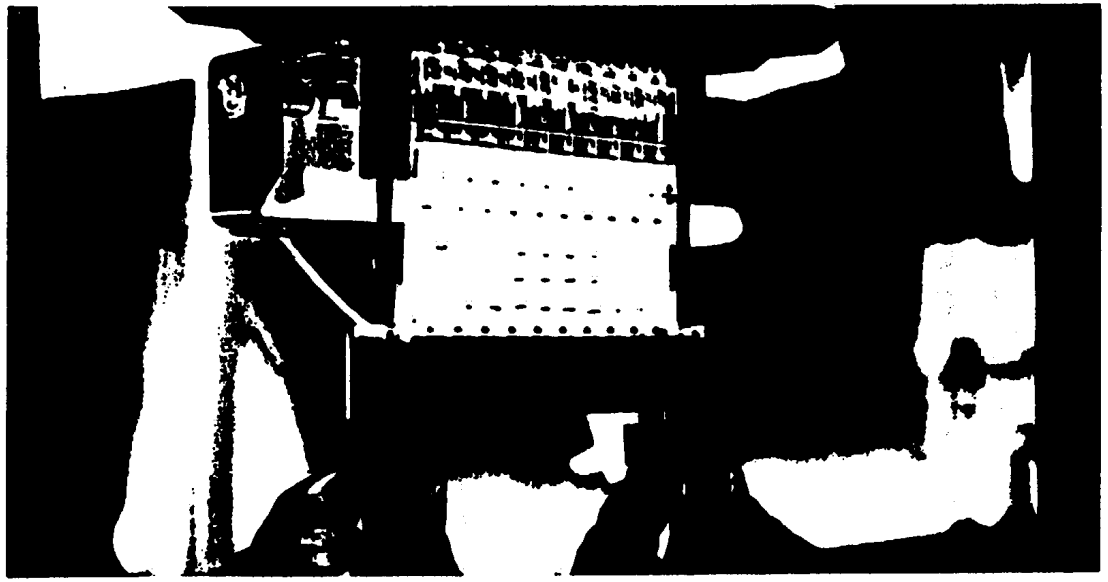
FIGURE 5: Time series plot representing methoprene residues found over a period of one week in a burley tobacco processing plant.



Methoprene, R = isopropyl
MPM, R = sec-butyl

Fig. 1

Fig 2



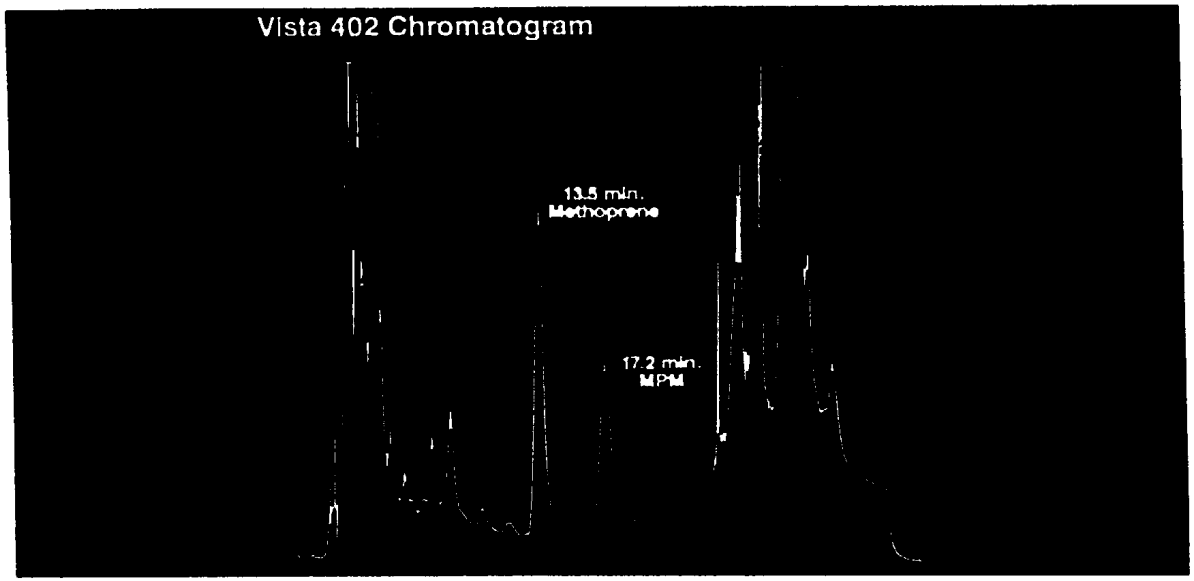


Fig. 3

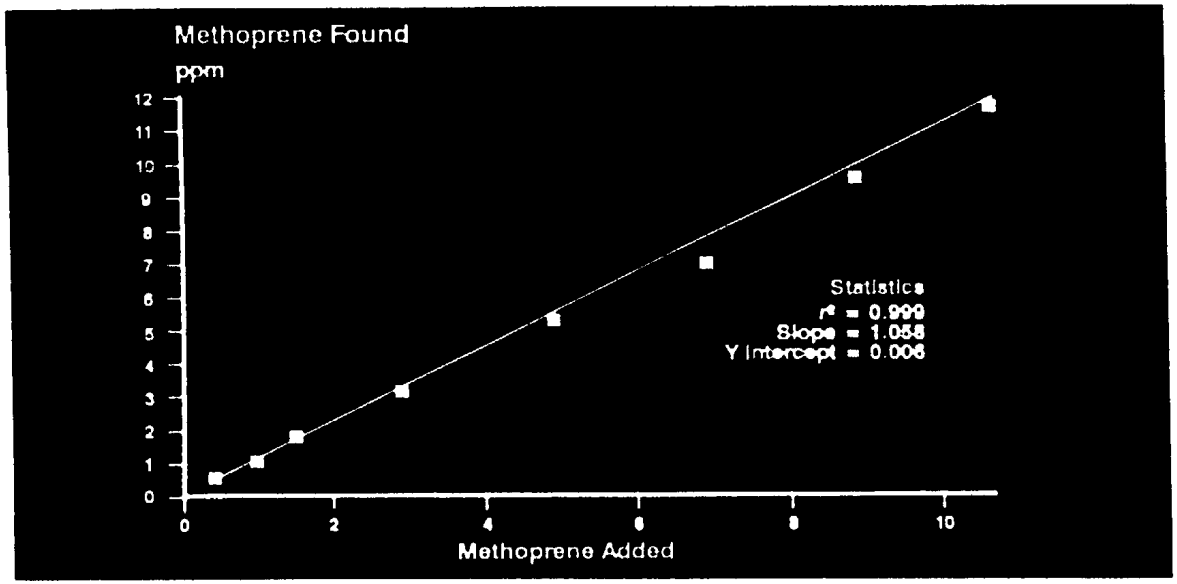


FIG. 4

Fig 5