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Liquid chromatographic method for the analysis of two plant based insecticide synergists dillapiole and dihydrodillapiole

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Abstract

A reversed-phase LC method for the determination of two plant based insecticide synergists dillapiole (5-allyl 6,7-dimethoxy 1,3-benzodioxole) and dihydrodillapiole (5-*n*-propyl 6,7-dimethoxy-1,3-benzodioxole) is reported. The resolution of dillapiole and dihydrodillapiole has been achieved on RP-18 column using methanol–water (90:10, v/v) as mobile phase and a photodiode array detector at 207 nm. The response was linear in the range of 25–250 μg. The developed isocratic RP-LC method was validated for specificity, linearity, precision, and accuracy. It has been applied for individual or simultaneous detection, monitoring and quantification of dillapiole and dihydrodillapiole from treated French bean *Phaseolus* sp.

Keywords: *Anethum sowa*; Dillapiole; Dihydrodillapiole; Pesticides

1. Introduction

Insecticide synergists play a significant role in enhancing the insect control potential of active ingredients by broadening their bioactivity spectrum, countering resistance development, increasing effective commercial lives, and mitigating the residual effects of persistent and highly toxic products by reducing application dose. Because of the multifarious uses, these have been particularly employed to formulate high cost insecticides such as natural pyrethrins. The introduction of synthetic pyrethroids during seventies sent the use of natural pyrethrins in oblivion. However, failure of these chemicals on several fronts has brought natural pyrethrins back into reckoning. This has also resulted in re-emergence of commercial interest in pyrethrum synergists.

Piperonyl butoxide (PBO), a commercial insecticide synergist containing a methylenedioxyphenyl and a polyalkoxy side chain as synergophoric groups has been in extensive use to synergise pyrethrins. It acts by inhibiting microsomal enzymes such as mixed function oxidases. Due to its suspected acute and chronic toxicity, interest in PBO is waning. The near at par synergistic activity of the plant based insecticide synergists dillapiole (5-allyl 6,7-dimethoxy-1,3-benzodioxole), a natural constituent of *Anethum sowa* Roxb. (Indian dill) [1] and the more stable reduced derivative dihydrodillapiole (5-*n*-propyl 6,7-dimethoxy 1,3-benzodioxole) [2] (Fig. 1) with PBO, has brought these two options to the fore and commercial interest in these materials has revived. Both the compounds can be used individually or in combinations to synergise insecticidal formulations. The gas chromatography–mass spectrometry (GC–MS) analysis of steam-distilled volatile dill oil showed the presence of 21 constituents of which dillapiole (15.92%) was the third major constituent [3]. In yet another GC–MS analysis, dillapiole has been found to be the second major constituent in the essential oils from the aerial parts of *Rutheopsis herbanica* (Bolle) Hans. & Kunk [4]. The major amount of dillapiole is confined in the heavier dill oil fraction generally considered as waste by the perfumery industry. A large number of dillapiole based synthetic and semi-synthetic compounds having superior synergistic activity are also known [5–10].

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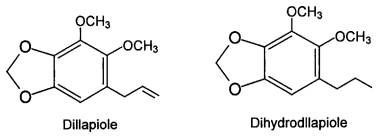

Fig. 1. Structures of dillapiole and dihydrodillapiole.

Certain amount of dillapiole left unhydrogenated during hydrogenation of dillapiole is likely to be present as an impurity in the dihydrodillapiole samples. An analytical method is therefore required which is capable of analyzing both dillapiole and dihydrodillapiole when present together. So far, dillapiole has been analyzed by gas chromatography and GC–MS [3,4]. Since liquid chromatographic (LC) methods are assuming increasing importance in pesticide analysis, the present paper reports a reversed-phase LC method for the individual or simultaneous analysis of dillapiole and dihydrodillapiole. This is incidentally the first report of analysis of dillapiole and dihydrodillapiole by HPLC.

2. Experimental

2.1. Reagents and solvents

HPLC grade water was prepared in laboratory by passing the city supply through a reverse osmosis (RO) unit and a water purification system (US Filter, Purelab classic). HPLC grade methanol (Qualigens India, a unit of GlaxoSmith-Kline) was procured. Dill oil was available from the local market. All solvents were degassed and filtered through a filtration system before use. Since standards of dillapiole and dihydrodillapiole were not available, their standard solutions were prepared by using purified dillapiole and dihydrodillapiole as standard samples.The purified sample of dillapiole was obtained by re-distillation of the pure dillapiole obtained after fractional distillation of dill oil. The purified dihydrodillapiole sample was obtained by distillation of the hydrogenated dillapiole. As revealed by LC, elemental analysis, ^{1}H NMR and mass spectral data, both dillapiole and dihydrodillapiole were found to be sufficiently (>95%) pure.

2.2. Isolation and characterization of dillapiole from dill oil

Dillapiole was obtained from dill oil by its fractional distillation. It distilled out at approx. 285 °C decomp., 110–148 °C/8 mm Hg [0.1547 psi or 1066.578 Pa (conversion factor 760 mm Hg = 14.7 psi = 101,325 Pa)], yield 40–60% [IR: $\nu_{max}^{CCl_4}$: 1615 (aromatic), 950 (OCH_2O), 840 cm^{-1} ($C{=}CH_2$); ^{1}H NMR δCCl_4: 3.15 (d, 2H, J = 6.5 Hz, $ArCH_2$), 3.59 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.87 (d, 2H, J = 10 Hz, $CH{=}CH_2$), 5.69 (m, 1H, $CH{=}CH_2$), 5.71 (s, 2H, OCH_2O), 6.05 (s, 1H, aromatic proton). Anal. calcd. for $C_{12}H_{14}O_4$: C, 64.9; H, 6.3. Found: C, 64.4; H, 6.3].

2.3. Preparation of dihydrodillapiole

Dihydrodillapiole was prepared by catalytic hydrogenation of dillapiole using palladised charcoal or Raney nickel as catalyst at 10 psi hydrogen pressure at ambient temperature [IR: peak at 840 cm^{-1} for $C{=}CH_2$ diminished. ^{1}H NMR δCCl_4: 0.94 (t, 3H, J = 6.5 Hz, CH_2CH_3), 1.52 (m, 2H, CH_2CH_3), 1.52 (m, 2H, OCH_3), 2.5 (t, 2H, J = 6 Hz, $ARCH_2$), 3.73 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 5.81 (s, 2H, OCH_2O), 6.33 (s, 1H, aromatic proton). Anal. calcd. for $C_{12}H_{16}O_4$: C, 64.3; H, 7.1. Found: C, 64.3; H, 6.6].

2.4. Preparation of standard solutions

Standard solutions of dillapiole and dihydrodillapiole (1000 ppm) were prepared by dissolving 10 mg of each in 10 mL of methanol. Working standard solutions for LC were prepared in the range of 1–250 μg mL^{-1} through a serial dilution of the standard solution with the mobile phase.

2.5. LC apparatus

Reverse phase LC system consisted of a Waters 600 quaternary pump with a manual injector (20 μL fixed loop), and a 996 photodiode array detector (Waters, USA). A computer using an "Empower" software programme integrated the peak areas automatically.

2.5.1. Chromatographic conditions

The chromatographic analysis was performed at ambient temperature (25–30 °C) on a Discovery HS C_{18} (250 mm × 4.6 mm i.d. 5 μm, Supelco, Sigma–Aldrich, USA); a 4 μm LiChrospher RP-select B (250 mm × 4 mm i.d., Merck, Darmstadt, Germany); RP-8: (250 mm × 4 mm i.d., Merck), and Chromolith (100 mm × 4.6 mm i.d., Merck) columns. The mobile phase comprised of isocratic mixture of methanol–water (95:5; 90:10; and 80:20, v/v) at flow rates of 0.4, 0.5, and 0.75 mL min^{-1}. For quantification, the photodiode array detector was set at 207 nm. A 20 μL portion of sample was injected each time.

2.6. Extraction, clean up and recovery of dillapiole and dihydrodillapiole from French bean (Phaseolus sp.)

Twenty-five gram each portions of untreated French bean samples were spiked separately in triplicate with known quantities of solutions of dillapiole and/or dihydrodillapiole in acetone to obtain concentrations of 62.5, 125 and 250 μg. These were extracted in 100 mL acetone and the extract passed through a column containing layers of activated Florisil and neutral alumina. The organic phase was removed under reduced pressure using a rotary evaporator at 40–45 °C. The residue was dissolved in LC grade methanol

and volume made to 10 mL. A 20 μL aliquot of this solution was injected and chromatographed. Authentic samples of known concentration of each component were used as reference. For simultaneous determination of both dillapiole and dihydrodillapiole a 25 g portion of the vegetable sample was fortified with their mixture, extracted and chromatographed as described above. All treatments were done in triplicate. Percentage recovery was calculated based on the difference between experimental and calculated values as follows:

$$\text{recovery (\%)} = \frac{\text{component measured}}{\text{component added in spiked vegetable (bean)}} \times 100$$

The concentration of each compound in the extracted vegetable sample was calculated by comparing the peak area with standard as follows:

$$C_i = \frac{A_i}{K}$$

where C_i is the concentration of the component i, A_i is the area of the peak corresponding to i, and K, the peak area of the standard/concentration of standard.

3. Results and discussion

3.1. Method development

Structurally, dillapiole and dihydrodillapiole differ from each other only in the former having a double bond in the alkenyl side chain. Reversed-phase RP-C_{18} or C_8 bonded phase columns were chosen for the analysis. Different mobile phase compositions were tried to optimize resolution time. Physico-chemical properties of the stationary phases used in different columns are reported in Table 1. Columns with 100–250 mm length, particle size of 3–5 μm, carbon loading of 11.5–21.6%, medium surface area of 300–360 $m^2\ g^{-1}$, and a pore size of 60–130 Å were tested. A binary mobile phase of different compositions containing methanol–water (95:5; 90:10; 80:20, v/v) was used. Only Discovery HS C_{18} and RP select B columns were found to give good separation in a reasonable run time (Fig. 2). With Chromolith and RP-18e columns, dillapiole and dihydrodillapiole eluted within 15 min as a single peak with tailing.

Discovery HS C_{18} and RP select B were optimized for solvent mixture composition, flow rate, and limits of detection and quantification (Tables 3 and 4). Discovery HS C_{18} column, methanol–water (90:10) at flow rate of 0.5 mL min^{-1} were found optimum for the analysis.

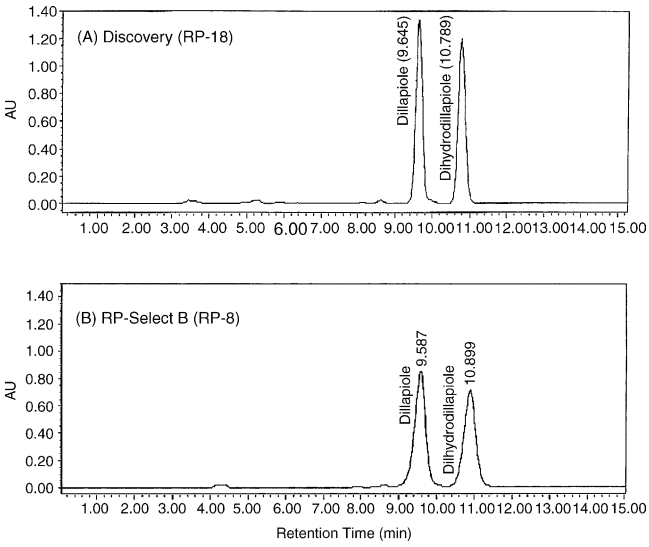

Fig. 2. Reversed-phase LC separation of dillapiole and dihydrodillapiole (50 μg mL^{-1} each) on a Discovery HS C_{18} (RP-18) (A); and RP-Select B column (B); using isocratic solvent system MeOH–water (90:10, v/v); at flow rate 0.50 mL min^{-1}, and UV detection at 207 nm.

Table 2
Accuracy of the method for LC determination of dillapiole and dihydrodillapiole

Compound	Concentration (ppm)	Mean found (ppm)	(±) S.D. (ppm)
Dillapiole	25	24.20	0.27
	50	48.84	0.24
	100	92.78	0.35
Dihydrodillapiole	25	24.16	0.26
	50	49.17	0.35
	100	90.19	0.52

3.2. Method validation

3.2.1. Accuracy

Five-fold injections of 25, 50, 100 ppm of dillapiole and dihydrodillapiole were used to determine the accuracy and standard deviation. The accuracy of this method for the determination of both the synergists (Table 2) was found to be adequate.

Table 1
Physico-chemical properties of LC columns used in the study

Column	Length and i.d. (mm)	Particle size (μm)	Pore size (Å)	Carbon load (%)	Coverage (μmol m^{-2})	Surface area ($m^2\ g^{-1}$)
Discovery HS C_{18}	250 × 4.6	5	120	20.0	3.80	300
RP Select B	250 × 4.6	4	60	11.5	3.55	360
RP-18e	250 × 4.0	5	100	21.6	4.09	350
Chromolith	100 × 4.6	3	130	18.0	3.60	300

Table 3
Optimum parameters for the analysis of dillapiole and dihydrodillapiole by liquid chromatography using different solvent systems at flow rate of 0.5 mL min^{-1}

Solvent system (methanol–water)	Retention time (t_R, min)			
	Dillapiole		Dihydrodillapiole	
	t_R	Peak height	t_R	Peak height
Discovery HS C_{18} column				
95:05	9.124	2136004	9.964	2054697
90:10	9.645	2265339	10.789	2075700
80:20	15.934	2253648	19.474	2103658
RP Select B				
90:10	9.507	1602213	10.899	1345513

3.2.2. Precision

The injection precision, a measure of the method variability was determined by performing analysis of the same working solution in six replicates. R.S.D. of the results was used to evaluate precision of the method. Injection precision was determined for six replicate injections of a representative batch of both the compounds separately at the target concentration of 100 μg mL^{-1}. R.S.D.s of the response factor of dillapiole and dihydrodillapiole peaks were respectively 1.23 and 1.90% for the six injections, indicating a good precision of the method.

3.2.3. Linearity

Three calibration curves with five standard solutions each were prepared. Concentration range of 25–250 μg mL^{-1} was used with the target concentration of 100 μg mL^{-1} for both the compounds. The UV detector response for both the compounds was linear over this range with correlation coefficients of 0.9995 and 0.9924 for dillapiole and dihydrodillapiole, respectively. Peak area (y) and concentration (x) of each injection was subjected to regression analysis to calculate the calibration equation and correlation coefficients. Linearity was confirmed as the R.S.D. values of the slope (1.14 and 2.80) and the intercept (2.80 and 2.56) were less then 3%. Least-square regression calibration curves were constructed by plotting peak areas of dillapiole and dihydrodillapiole as a function of concentration in the standard working solutions. The calibration curves could be represented by the following regression equations:

$$y\,(\text{dillapiole}) = 0.031x + 0.2419 \quad (r = 0.9995, n = 5)$$

$$y\,(\text{dihydrodillapiole}) = 0.0262x + 0.3592 \quad (r = 0.9924, n = 5)$$

where 'x' is the concentration of dillapiole or dihydrodillapiole in μg mL^{-1} and 'y' the peak area ratio. The individual linear range was 24.75–250.94 μg mL^{-1} for dillapiole and 27.68–255.17 μg mL^{-1} for dihydrodillapiole. The results show that within the test concentration range, there was an excellent correlation between peak area and concentration of both the synergists.

3.2.4. Recovery

The optimized LC conditions were applied to the determination of dillapiole and dihydrodillapiole in fortified French bean samples, first individually and then simultaneously. Bean samples spiked with 25, 50 and 100 μg g^{-1} of vegetable sample were used in triplicate to assess accuracy. Negligible clean up was used. The amounts of dillapiole and dihydrodillapiole were calculated from related linear regression equations. The recoveries are presented in Table 4. The vegetable blank did not show any peak interfering with those of dillapiole and dihydrodillapiole. The percentage recovery ranged from 88.5 to 92.3% with R.S.D. values less than 0.84%, and 91.0 to 96.4% with R.S.D. values less than 1.00%, for dillapiole and dihydrodillapiole, respectively (Table 5). Good recoveries achieved from vegetable samples indicated that the method has sufficient accuracy for the determination of the active ingredient(s) either alone or in mixture.

Table 4
Optimum parameters for the analysis of dillapiole and dihydrodillapiole by liquid chromatography using methanol–water (90:10) as eluent

Solvent flow rate (mL min^{-1})	Retention time (t_R, min)			
	Dillapiole		Dihydrodillapiole	
	t_R	Peak height	t_R	Peak height
Discovery HS C_{18} column				
0.40	12.013	2204589	13.439	2008961
0.50	9.645	2265339	10.789	2075700
0.75	6.486	2310569	7.247	2201578
RP Select B				
0.50	9.507	1602213	10.899	1345513

Table 5
Percent recovery of dillapiole and dihydrodillapiole from French bean

Compound	Added concentration ($\mu g\,mL^{-1}$)	Recovered concentration ($\mu g\,mL^{-1}$)	R.S.D. (%)	Average recovery (%)
Dillapiole	25	22.8	0.86	91.2
	50	48.7	0.45	97.4
	100	89.6	0.69	89.6
Dihydrodillapiole	25	25.4	0.63	101.6
	50	48.5	0.92	97.0
	100	91.8	0.77	91.8

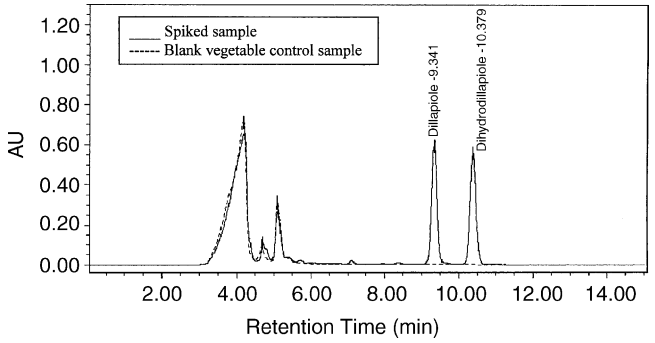

Fig. 3. LC chromatogram of bean sample (—) spiked with 25 $\mu g\,mL^{-1}$ each of dillapiole and dihydrodillapiole, (- - -) blank vegetable control sample.

Specificity of the HPLC method is illustrated in Fig. 3. A complete separation of dillapiole and dihydrodillapiole from the extracted biological endogenous components in bean was noticed. No interfering peaks at the retention times of dillapiole and dihydrodillapiole were observed.

3.2.5. Limit of detection (LOD) and quantification (LOQ)

Detection limit is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated. The lowest limit is usually evaluated as the signal-to-noise ratio that is equivalent to three times the standard deviation of the noise (S/N = 3). Limits of detection, and quantification (LOD and LOQ) were estimated in accordance with the baseline noise, which was evaluated by recording the detector response over a period of as much as 10 times the peak width. The lower limit of detection for dillapiole and its dihydro-derivative was established as 2.84 and 4.85 $\mu g\,mL^{-1}$ as their 20 μl injection gave a measurable peak. The limit of quantification (LOQ), which is defined as the lowest concentration that can be determined with acceptable accuracy and precision, can be established at a signal-to-noise ratio of 10. Limits of quantification of both dillapiole and dihydrodillapiole were experimentally verified by six injections and were found to be 9.47 and 16.17 $\mu g\,mL^{-1}$, respectively (Table 6).

Results on the effect of nature of the stationary phase, particle size, carbon load, surface area and mobile phase using Discovery HS C_{18} and RP select B column in RP-HPLC separations are reported in Table 2; the former column was distinctly more favourable than the latter with significant difference in detection and quantification.

Table 6
Limits of detection and quantification ($\mu g\,mL^{-1}$) of dillapiole and dihydrodillapiole on two reversed-phase columns

Column	Dillapiole		Dihydrodillapiole	
	LOD	LOQ	LOD	LOQ
Discovery HS C_{18}	2.84	9.47	4.85	16.17
RP Select B	4.00	13.35	7.23	24.10

4. Conclusions

It is very likely that a plant based insecticide synergist dillapiole, its more stable saturated analogue dihydrodillapiole or their combination may replace commercially available synthetic and toxic insecticide synergist piperonyl butoxide to synergise pyrethrum. An isocratic reverse phase LC method has therefore been developed for their analysis. It enables simultaneous determination of the two analytes, of close polarities and chromatographic behavior. The proposed LC method has been verified for accuracy, precision, and selectivity. It requires a short time (<1 h) for sample analysis including sample clean-up procedure. Involving use of Discovery HS C_{18} column and an isocratic mobile phase, it offers improved resolution and increased peak height, and eliminates the need for gradient separation. The two synergists could be separated and determined in less than 15 minutes. The method is recommended for the routine assay of plant origin dillapiole and dihydrodillapiole alone, in combinations or in synergised pesticidal formulations.

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