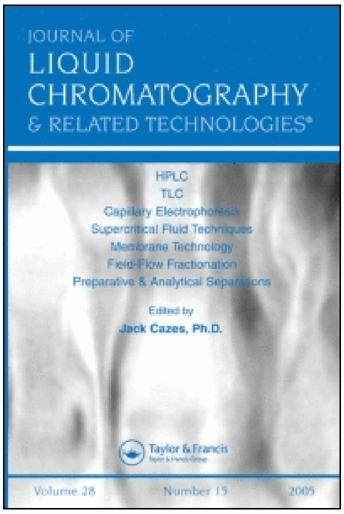

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SIMULTANEOUS DETERMINATION OF DIPROPYL PYRIDINE-2,5-DICARBOXYLATE, N-OCTYL BICYCLOHEPTENE DICARBOXIMIDE, PIPERONYL BUTOXIDE, AND PYRETHRINS IN PET SHAMPOO BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of six pyrethrin esters, piperonyl butoxide (PBO), dipropyl pyridine-2,5-dicarboxylate (MGK 326), and endo- and exo-N-octyl bicycloheptene dicarboximide (MGK 264) in pet shampoo formulations. A C_8 Octyl reverse phase column and different compositions of acetonitrile, methanol and water as mobile phases were used. The eluent was monitored with UV absorption at 220, 230, 240 and 225 nm. Internal standard calculations using peak area was used to calculate each active ingredient peak. Interfering inert ingredients were separated from all active ingredients using this method. This simple and reliable technique has an advantage over GC because of the thermal degradation of pyrethrins, and over normal phase high performance liquid chromatography, since it requires no further sample clean-up.

INTRODUCTION

Pyrethrins and PBO or MGK 264 as a synergist are commonly used insecticide combination formulations.[1] Several publications have described the determination of pyrethrins, PBO and MGK 264 in various formulations by high performance liquid chromatography (HPLC)[2-12] or by gas chromatography (GC).[13-16] HPLC methods are preferred to GC methods for analyzing pyrethrins and related compounds due to the thermal instability of pyrethrins.[17-18]

There is little mentioned in the literature concerning the simultaneous determination of six pyrethrin esters, PBO and MGK 264 in products formulations by reverse phase HPLC methods without a sample clean-up step. Bushway[9] used normal phase liquid chromatography to determine six pyrethrin esters in formulations after a sample extraction procedure. PBO and MGK 264 were not quantitated and resolved by their procedure.

With the aid of state-of-the-art columns and HPLC equipment, the optimized reverse phase HPLC method can be developed and optimized in complex systems.[19-21] By changing the selectivity factor (α), the resolution (R_s) can be improved. This gives us a powerful tool to improve separation and decreasing analysis time.

Large changes in reverse phase HPLC selectivity can be achieved by using two organic solvents in ternary mobile phases, by changing temperature, or by using gradient elution.

$$R_s = (1/4)(\alpha\text{-}1)\sqrt{N}\ (K'1 + K')$$

where α = selectivity factor,
N = efficiency factor and
K' = retention factor

This paper describes, in detail, the development and optimization of a reverse phase HPLC method using ternary mobile phases that allows the rapid and simultaneous analysis of six pyrethrin esters, endo- and exo-MGK 264, MGK 326, and PBO in pet shampoo formulations without a sample extraction procedure. By this methodology, the six pyrethrin esters; "pyrethrin I" (jasmolin I, cinerin I, and pyrethrin I), "pyrethrin II" (jasmolin II, cinerin II, and pyrethrin II), PBO, MGK 326, and endo- and exo-MGK 264 (Figure 1) can be separated and quantified.

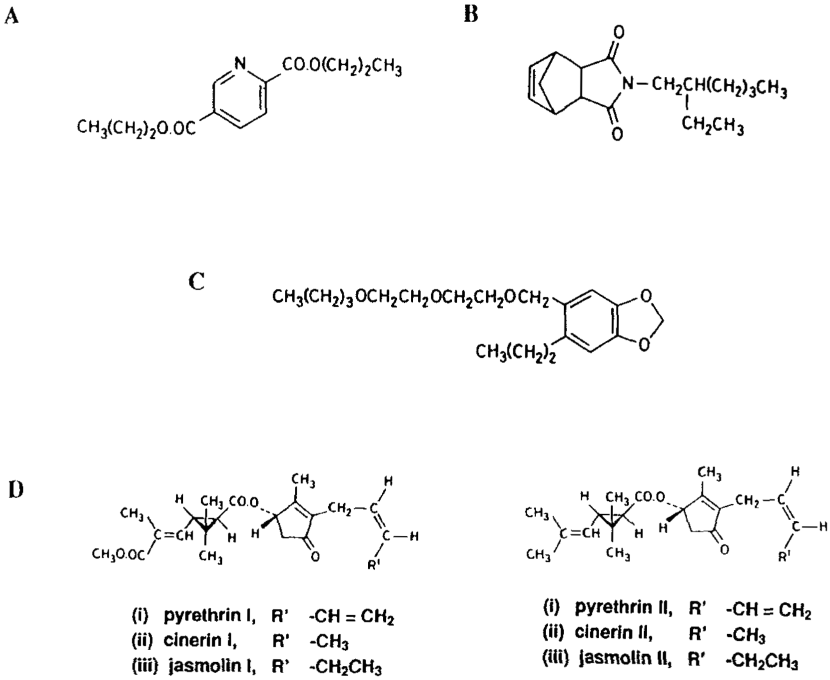

Figure 1. Structures of the active ingredients of pet shampoo. A, MGK326; B, MGK264; C, Piperonyl butoxide; D, Pyrethrins.

EXPERIMENTAL

Instrumentation and Data Analysis

A Hewlett-Packard 1050 high performance liquid chromatography equipped with a variable wavelength-diode array detector, online degasser, heated column compartment, and automatic sampler and injector was used.

The chromatographic peaks and data analysis were recorded by employing a Hewlett-Packard 3357 laboratory automation system. The HPLC column used was a 150 mm × 4.6 mm I.D., 5 μm Restek Pinnacle C_8 Octyl reverse phase column.

Chemicals

Shampoo samples of Lot #119711-01 and Lot #119731-01 were provided by QC Department, Sandoz Agro, Inc. (Dallas, TX). Analytical standards of pyrethrum, PBO and MGK 264 were obtained from Sandoz Agro, Inc. (Dallas, TX). Analytical standard MGK 326 was obtained from McLaughlin Gormley King Co. (Minneapolis, MN). Internal standard octanophenone is available from Aldrich Chemical Company (Milwaukee, WI). Acetonitrile, methanol, isopropyl alcohol and water solvents were purchased from Fisher Scientific and were HPLC grade.

Reverse Phase HPLC Methodology

To achieve the best separation, chromatography was carried out at 35 °C. A three-component mobile phase (methanol, acetonitrile, and water) was used for gradient elution. The volume ratios of the solvents were according to the following time table.

Time (min)	% Water	% Acetonitrile	% Methanol
0	52	38	10
12	52	38	10
16	42	48	10
20	42	48	10
23	42	58	0
25	35	65	0
30	15	85	0
35	15	85	0

The injection volume was 20 µl of a 5 gram of pet shampoo sample that was dissolved in 100 ml of isopropyl alcohol. The internal standard method (using octanophenone as the internal standard) is employed.

A constant flow rate of 0.8 ml/min was used. Diode array detection is selectively performed at 230 nm (0 min), 220 nm (18 min), 240 nm (26 min) and 225 nm (31.5 min).

Table 1

UV Absorption Data for the Internal Standard Octanophenone, MGK 326, MGK 264, PBO, and Pyrethrins in the Methanol, Acetonitrile and Water Mixtures

Compound	**λ max(nm)**
Octanophenone	242
MGK 326	231
exo-MGK 264	198
endo-GMK 264	190
PBO	238
Cinerin I	223
Jasmolin I	228
Pyrethrin I	225
Cinerin II	235
Jasmolin II	235
Pyrethrin II	229

RESULTS AND DISCUSSION

Characteristics of the HPLC chromatograms

The UV maximum absorption of the individual peaks eluted from the reverse phase HPLC are listed in Table 1. These active ingredients were analyzed individually and the results were used to identify active ingredients in pet shampoo samples. Figure 2 shows typical chromatograms of analytical standards and internal standard, shampoo placebo sample (without adding any active ingredients), and pet shampoo sample. The six pyrethrin esters (cinerin II, pyrethrin II, jasmolin II, cinerin I, pyrethrin I, and jasmolin I), endo- and exo-MGK 264, MGK 326, and PBO were separated from the internal standard and shampoo inert ingredients. The retention time of each active ingredient is listed in Table 2. UV diode array detection was used to check the peak purity and to confirm the analyte peak identity. The use of methanol solvent in the mixtures of acetonitrile and water mobile phases successfully separated pyrethrin II group from exo-MGK 264 , and from the inert ingredients without increasing the separation time. By this method pyrethrin II group was also separate from the internal standard octanophenone.

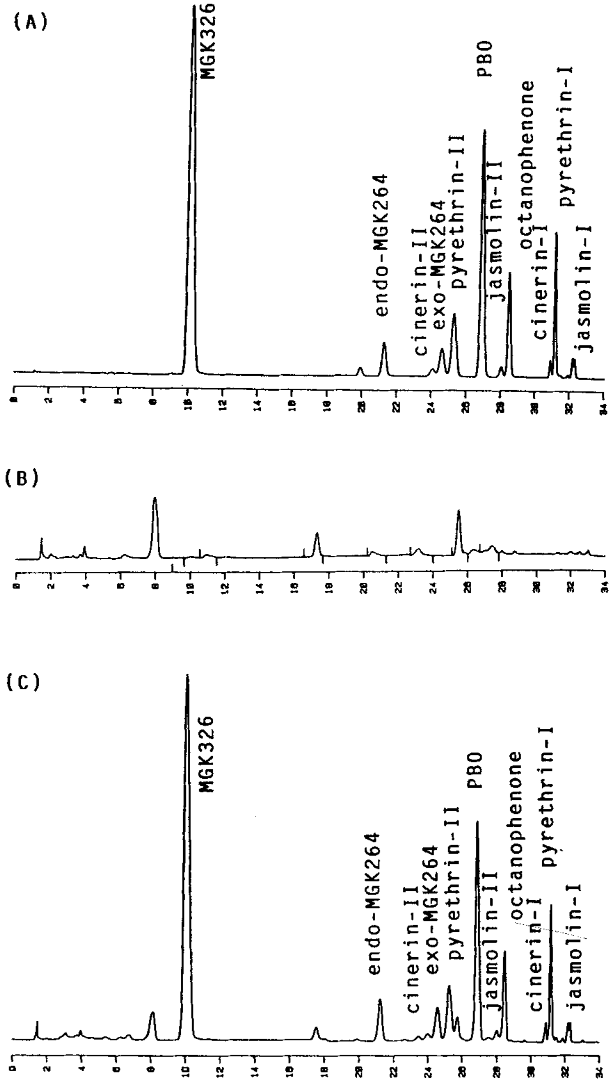

Figure 2. HPLC chromatograms of (A) analytical standards and internal standard, (B) a placebo shampoo sample and (C) a pet shampoo sample in isopropyl alcohol.

Table 2

Experimental Results for the Retention Time and Recoveries of MGK326, MGK264, PBO and Six Pyrethrin Esters in Pet Shampoo Samples by Reversed Phase HPLC

Compound	Retention Time (min)	Amount Spiked (mg)	Amount Found (mg)
MGK326	10.12	6.36	6.61
		12.71	13.81
		19.06	20.15
		26.48	27.47
endo-MGK264	21.24	3.25	3.36
		6.51	6.97
		9.76	10.17
		13.56	13.73
Cinerin-II	24.02	0.35	0.39
		0.70	0.76
		1.06	1.13
		1.47	1.50
exo-MGK264	24.57	2.80	2.84
		5.60	5.86
		8.40	8.72
		11.66	11.78
Pyrethrin-II	25.27	0.24	0.25
		0.48	0.52
		0.73	0.76
		1.01	1.03
PBO	26.87	12.79	12.76
		25.57	26.68
		38.36	39.50
		53.27	53.21
Jasmolin-II	28.01	0.19	0.22
		0.38	0.43
		0.57	0.63
		0.79	0.84
Cinerin-I	30.88	0.23	0.24
		0.46	0.49
		0.68	0.71
		0.95	0.96

(continued)

Table 2 (continued)

Compound	Retention Time (min)	Amount Spiked (mg)	Amount Found (mg)
Pyrethrin-I	31.15	0.66	0.64
		1.31	1.37
		1.97	2.02
		2.73	2.71
Jasmolin-I	32.31	0.16	0.18
		0.32	0.34
		0.48	0.50
		0.67	0.68

Quantitative Study

Regression analysis of reverse phase HPLC determination of six pyrethrins, endo-and exo-MGK 264, MGK 326 and PBO in isopropyl alcohol

The linearity of the developed method was verified. Various concentrations of analytical standards and internal standard in isopropyl alcohol were analyzed to prepare the calibration curves. Calibration graphs were constructed by plotting the injected amount for each active ingredients as a function of peak area. The corresponding regression lines were fitted using the least-squares method. Statistical evaluation of the HPLC analysis are listed in Table 3. The linear regression equations and correlation coefficients showed that the standard curves were linear in the investigated area.

Linearity of chromatographic responses of MGK 326, MGK 264, PBO and pyrethrin esters in pet shampoo samples measured by standard additions method

The possible presence of interferences due to the shampoo inert ingredients and active ingredient impurities was examined by comparison of the linear equations for the results obtained by the standard additions method on pet shampoo samples. The equal amounts of shampoo samples were spiked known amounts of analytical standards. These spiked amounts were at various concentrations according to the described procedure. The HPLC data were then determined for all these solutions. The linear regression equations and correlation coefficients are listed in Table 4. The intercepts ($x = 0$) which are

Table 3

Statistical Evaluation of the Reversed Phase HPLC Analysis

Compound	Regression Equation	Correlation Coefficient
Octanophenone	y = 2382.5x - 617.21	0.9997
MGK 326	y = 7577.4x - 25950	0.9993
PBO	y = 1468.9x - 6516.3	0.9994
endo-MGK264	y = 135.38x + 1024	0.9995
exo-MGK264	y = 106.65x - 1136.3	0.9984
Pyrethrin I	y = 14810x - 2644.8	0.9994
Pyrethrin II	y = 57287x - 20233	0.9989
Cinerin I	y = 5311x + 1071.7	0.9995
Cinerin II	y = 5324.9x - 4211.3	0.9990
Jasmolin I	y = 7450x - 538.21	0.9992
Jasmolin II	y = 587.1x - 1233	0.9997

Table 4

Statistical Data for the Method of Standard Additions

Compound	Regression Equation	Correlation Coefficient
MGK 326	y = 108599x + 4E+06	0.9979
PBO	y = 24818x + 1E+06	0.9988
endo-MGK264	y = 1721x + 327953	0.9993
exo-MGK264	y = 18183x + 207764	0.9984
Pyrethrin I	y - 134618x + 392666	0.9977
Pyrethrin II	y= 380201x + 315297	0.9991
Cinerin I	y - 48445x + 64461	0.9965
Cinerin II	y = 17986x + 25153	0.9987
Jasmolin I	y = 75893x + 48429	0.9974
Jasmolin II	y = 46038 x + 26986	0.9980

equal to the sample without spiked are very close to the values of HPLC experimental data (Table 5). The unweighed regression lines can be obtained by extrapolating back to the point on the x-axis at which $y = 0$. The negative intercept on the x-axis corresponds to the amount of the active ingredients in the formulations.

Table 5

Results of Standard Additions for the Determination of Unknown Active Ingredients in Pet Shampoo Formulations by Reversed Phase HPLC Method

Compound	Calculated (Intercept)* Area Count	Experimental Area Count
MGK 326	4000000	3917707
PBO	1000000	1128257
endo-MGK264	327953	323066
exo-MGK264	207764	205505
Cinerin II	25153	25019
Pyrethrin II	315297	310101
Jasmolin II	26986	26903
Cinerin I	64461	63802
Pyrethrin I	392666	387393
Jasmolin I	48429	48435

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* From regression equations in Table 4.

These experiments also provided recovery data. The data were obtained by the analysis of the various amounts of spiked pet shampoo samples, quantified by the internal standard method. Recovery data determined by comparing the found and spiked amounts are reported in Table 2. Recoveries ranging from 100 to 104% were obtained for pet shampoo samples. The spiked amount ranging were from 120% to 200% of individual active ingredients in shampoo samples.

CONCLUSION

A reverse phased HPLC method for a relatively quick simultaneous determination of six pyrethrin esters, PBO, endo- and exo-MGK 264, and MGK 326 in pet shampoo formulations has been described. The developed HPLC method allows for analysis without the time consuming extraction pretreatment and filtration of the samples. The applicability of the method was demonstrated by recovery studies for real samples. The separation is performed in 35 min and the method yields accurate results and shows a good correlation.

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