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Isocratic reversed-phase liquid chromatographic method for the simultaneous determination of (*S*)-methoprene, MGK264, piperonyl butoxide, sumithrin and permethrin in pesticide formulation

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Abstract

An isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method development for the simultaneous determination of five active ingredients (*S*)-methoprene, MGK264, PBO, sumithrin and permethrin in a new complex pesticide formulation is described. These active ingredients have similar polarities and contain isomers. The RP-HPLC method development approach began with the selection of a column based on the component structure information, bonded phase, and particle physical characteristics. Second, the mobile phase composition was changed to improve peak resolution and peak sensitivity, especially with analytes containing isomers. Choosing the match between the stationary phase and mobile phase composition, the developed RP-HPLC method not only can simplify the procedure appreciably but also significantly decrease total analysis time and increase peak height. The developed isocratic RP-HPLC method for the analysis of this new formulation was then validated for specificity, linearity, precision, and accuracy. The chromatographic peak identification was identified by LC–MS using the electrospray ionisation in the positive-ion mode.

Keywords: Pesticides; Methoprene; MGK264; Piperonyl butoxide; Sumithrin; Permethrin

1. Introduction

Pyrethrum, pyrethroids, and their synergists such as PBO (piperonyl butoxide) and MGK264 (*N*-octylbicycloheptene dicarboximide) comprise an important group of insecticides [1,2]. The separation of pesticides by gas chromatography has been reviewed [3–5]. Recently, high-performance liquid chromatography (HPLC) and hyphenated techniques such as LC–MS and LC–MS–MS are increasingly being used, especially in pharmaceutical and biotech areas. Most such applications are performed on reversed-phase HPLC (RP-HPLC) on nonpolar C_8 or C_{18} bonded silica-based supports because of their selectivities and sensitivities for a large range of compounds [6]. RP-HPLC is also widely used in both analytical and preparative separations. Therefore, RP-HPLC becomes the most important separation mode in HPLC [7]. Several results using RP-HPLC techniques for the analysis of pesticide formulations have been reported by our laboratory [8–10]. The goals of RP-HPLC method development include in achieving high resolution, minimum amount of runtime, and retention time reproducibility. Resolution

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is affected significantly by the column bonded-phase, solvent composition, gradient elutions, flow-rate, buffer type, pH, and column temperature [11]. However, it is a time-consuming trial-and-error approach to develop new methods. With new developments in HPLC column materials based on packing morphology, design, bonded-phase chemistry and column construction, column selection can play an important role in RP-HPLC method development [12–14]. The mobile phase composition has a major effect on improving resolution and runtime, especially for the analysis of isomers. Therefore, method development approaches based on column selection and mobile phase optimization can result in simplifying analytical procedure and saving time in the laboratory.

Knowing the analyte component structures is a guide in choosing the column bonded-phase. The active ingredients in this pesticide formulation are permethrin, sumithrin, (*S*)-methoprene, PBO, and MGK264; their structures are shown in Fig. 1. Permethrin and sumithrin both have *cis*- and *trans*-isomers. (*S*)-Methoprene, an insect growth regulator, contains about 98% *trans*-isomer and 2% of *cis*-isomer. Synergist PBO is about 93% pure. Nitrogen containing sumithrin synergist MGK264 has endo- and exo-configurations [15]. The objective of this work was to develop an isocratic RP-HPLC method for the simultaneous determination of five active ingredients permethrin, sumithrin, (*S*)-methoprene, PBO, and MGK264 in a new commercial product of pesticide formulation. Using column selection and mobile phase optimization together achieves rapid analysis and high resolution in this method development. The developed isocratic RP-HPLC method was validated for specificity, linearity, precision, and accuracy. The chromatographic peak identifications were determined using LC–ESI-MS.

2. Experimental

2.1. Chemical and samples

Analytical standards of permithrin, sumithrin, (*S*)-methoprene, PBO, and MGK264 were obtained from Wellmark (Dallas, TX, USA). Placebo and pesticide formulations came from Wellmark's formulation department. The internal standard (I.S.) 2,2-dimethylpropiophenone was purchased from Aldrich (Milwaukee, WI, USA). Isopropyl alcohol, acetonitrile, methanol, and water were of HPLC grade from Fisher (Fairlawn, NJ, USA).

2.2. Sample preparation

Sample solutions for the precision test were prepared by dissolving 5 g of a pesticide formulation sample in 100 ml of an isopropyl alcohol (IPA) solution that contained 5 ml of the 0.8% of 2,2-dimethylpropiophenone I.S. solution. The accuracy of the method was evaluated by analyzing placebo samples spiked with known quantities of analytical pesticide standards at the level of ±50% of the target amounts. The accuracy test was carried out by running six different samples with three different standard solutions in triplicate. To prepare stock standard solutions for the linearity test, the analytical standards were accurately weighed in a placebo sample or in IPA. Subsequently the solutions were diluted with IPA to obtain seven working solutions that cover the expected 0–200% range of target values. For each analyte linear regression analysis was performed by plotting the peak area against the corresponding value. All sample solutions were well shaken and filtered through 0.45-μm polytetrafluoroethylene (PTFE) membrane filters made by Whatman (Clifton, NJ, USA) before injecting the solution into the HPLC column.

2.3. RP-HPLC analysis

The experiments were performed on Hewlett-Packard 1050 and Agilent 1100 Series HPLC (Palo Alto, CA, USA) both equipped with quaternary pump, autosamplers, column compartment, vacuum degasser, and diode-array detection systems. The instrument control and data processing utilities included the use of Hewlett-Packard application softwares CHEMSTATION and CHEMSERVER. All analyses were performed at a flow-rate 1 ml/min with detection at a wavelength of 240 nm. The injection volume was 10 μl and the column temperature was 35 °C. The columns used were Ace C_8 150×4.6 mm I.D., 3μm (MAC MOD, Chadds Ford, PA, USA) and Zorbax Eclipse XDB C_8 150×4.6 mm I.D., 5 μm (Agilent,

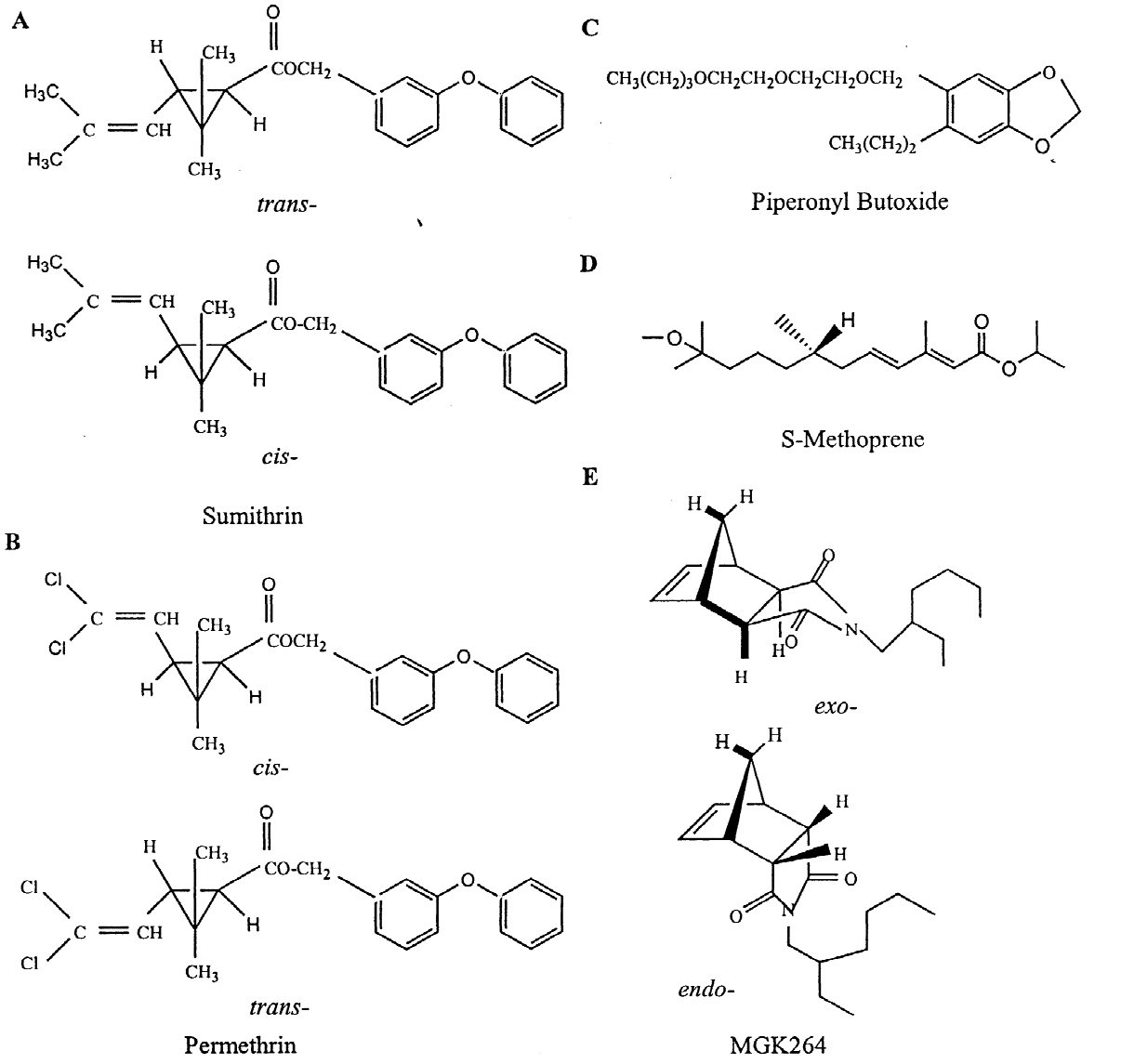


Fig. 1. Molecular structures of Sumithrin (A), Permithrin (B), PBO (C), (*S*)-Methoprene (D), and MGK264 (E) in the pesticide formulation.

Wilmington, DE, USA). The ternary mobile phase comprised methanol–water–acetonitrile (10:35:55, v/v/v).

2.4. LC–ESI-MS analysis

LC–MS was performed on an Agilent 1100 series LC–MSD system (Palo Alto, CA, USA) equipped with a binary solvent pump, an autosampler, vacuum degasser, a quadrupole MS detector consisting of standard positive and negative ionization modes, and an electrospray ionization (ESI) source. The chromatographic separation was achieved isocratically with the mobile phase containing water–methanol–acetonitrile (35:10:55), where the water–methanol contained 1.0 m*M* formic acid. The flow-rate was 0.8 ml/min, and the column temperature was maintained at 35 °C. The analytical column was an Agilent

Zorbax Eclipse XDB C_8 150×4.6 mm I.D., 5 μm. The eluent was monitored at 240 nm. Mass spectra were acquired in positive ion ESI (+). The ESI source conditions in PI mode were as follow: vaporizer temperature, 350 °C; nebulizer (nitrogen) pressure, 60 p.s.i. (1 p.s.i.=6894.76 Pa); drying gas (nitrogen) flow-rate, 10 l/min; drying gas temperature, 350 °C; capillary voltage, 4000 V; and the fragmentor voltage, 40 V. For each compound, the protonated molecular ion, $[M+H]^+$, and at least one confirming ion were acquired.

3. Results and discussion

3.1. RP-HPLC method development

Fig. 1 shows that these active ingredients are all apolar compounds with slight differences in being less or more apolar. The polarities of MGK 264 and PBO are higher than the polarities of other ingredients. All components have isomers except PBO. These isomers generally do not differ in polarity. Based on the structural information, either RP-C_{18} or C_8 bonded phase would be a good choice for this analysis. To improve resolution and decrease run time, an increase in the polarity of the stationary phase was needed. The less hydrophobic resolving power of RP C_8 bonded phase was used. Resolution is dependent in part upon column length and particle size of the packing material (μm) [11,14]. In order to select a RP C_8 column for high resolution and fast analysis, several commercial available C_8 columns were evaluated. The particle physical characteristics of these C_8 columns used in this study are summarized in Table 1. The average analytical column for most applications is 150×4.6 mm, 5 μm, 100 Å pore size, 200 m^2/g surface area, 10% carbon load, monomeric bonding, and spherical particles [14]. To compromise the fast analysis and high resolution for the separation of these apolar isomers, C_8 columns with a short column (150 mm), a small particle size (3- or 5-μm), medium carbon loading (7–9%), medium surface-area silica (200–300 m^2/g), and a low pore size (80–100 Å) were tested. Mobile phase composition in an analysis can also effectively increase resolution and decrease runtime. A ternary mobile phase containing methanol–water–acetonitrile (10:35:55, v/v) was used in this study based on our previous results [8–10]. After testing the columns listed in Table 1, only Ace and Zorbax column gave good resolution and a reasonable runtime. The results of the effect of the nature of the stationary phase, particle size, carbon load, surface area, and mobile phase using Ace and Zorbax column in RP-HPLC separations are illustrated in Figs. 2 and 3; the column in Fig. 3 is distinctly more favourable than that in Fig. 2 providing almost the same resolution in a significantly shorter time. The retention times and maximum UV absorbance of these analytes are listed in Table 2.

3.2. RP-HPLC method validation

On the basis of the parameters discussed above, this RP-HPLC method was validated according to the guidelines [16–18]. Chromatographic method validation consisting of method specificity, linearity,

Table 1
Particle physical characteristics of RP-HPLC C_8 columns

RP C_8 column	Particle size (μm)	Pore size (Å)	Carbon load (%)	Bond phase coverage (μmol/m^2)	Surface area (m^2/g)
Zorbax Eclipse XDB (Agilent)	5	80	7.6	3.6	180
Pinnacle Octyl (Restek)	3	120	6.5	3.85	170
Luna (Phenomenex)	5	100	14.75	5.5	440
Discovery (Supelco)	3	180	7.3	3.4	200
Symmetry (Waters)	5	100	12	3.38	345
Ace (MAC-MOD)	3.5	100	9	NA	300
Partisphere Plus (Whatman)	5	120	6	7	160
Partisphere RTF (Whatman)	5	100	17	NA	NA

NA, no data available.

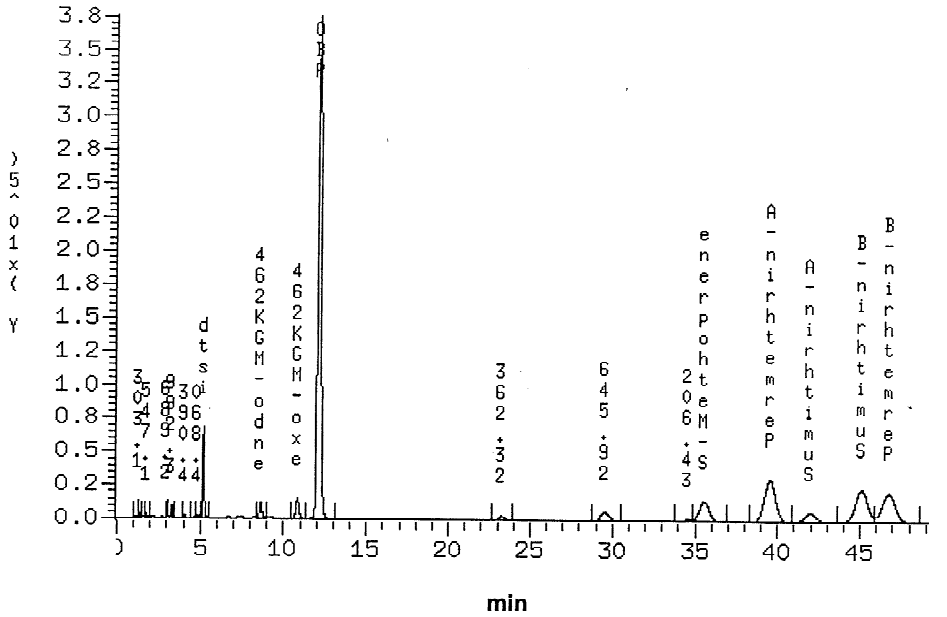


Fig. 2. RP-HPLC separation of a pesticide formulation on an Zorbax Eclipse XDB-C_8 column (150×4.6 mm, 5 μm) with isocratic mode MeCN–MeOH–H_2O (55:10:35, v/v/v); flow-rate 0.8 ml/min; 35 °C, UV 240 nm.

precision, and accuracy was undertaken in order to demonstrate the suitability of the analytical method for the determination of five pesticide active ingredients in this formulation.

3.2.1. Specificity

Specificity is a measurement of the degree of interference in the analysis of complex pesticide formulation. The placebo sample and each of the

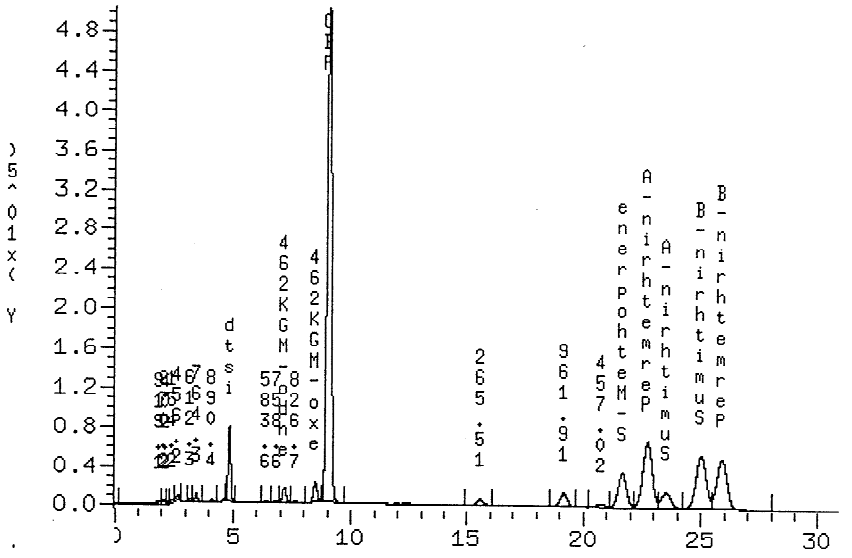


Fig. 3. RP-HPLC separation of a pesticide formulation on an Ace C_8 column (150×4.6 mm, 3 μm) with isocratic mode MeCN–MeOH–H_2O (58:10:32); flow-rate, 0.8 ml/min; 35 °C. UV 240 nm.

Table 2
Analytes and their HPLC retention times and maximum wavelength (λ_{max}) obtained on a Ace C_8 column eluted

Analyte	Retention time (min)	λ_{max} (nm)
ISTD	4.85	242
endo-MGK264	7.17	190
exo-MGK264	8.49	198
PBO	9.04	238
(*S*)-Methoprene	21.66	264
Permethrin-A	22.74	230
Sumithrin-A	23.54	225
Sumithrin-B	25.02	225
Permethrin-B	25.91	230

spiked active ingredient placebo samples were stored in an oven at 78 °C for 2 weeks before RP-HPLC analysis. The results showed that chromatograms presented the absence of interference from degradation products which is crucial to the targeted analytes of analytical method. The peak purity of stressed samples was evaluated by using HP CHEMSTATION purity calculation.

3.2.2. Response linearity

Six sets of six active ingredient standard solutions and internal standard solutions were carried out over the range of 0–200% of the target values. Data were collected from triplicate injections of each solution, and a plot was constructed of analyte peak area response versus active ingredient concentration (ppm). A linear regression was performed on each data set. Regress analysis showed an excellent linear relationship (r^2=1–0.9993) for all components. Fig. 4 shows the linearity plots for the chromatographic responses of (*S*)-methoprene, sumithrin, permethrin,

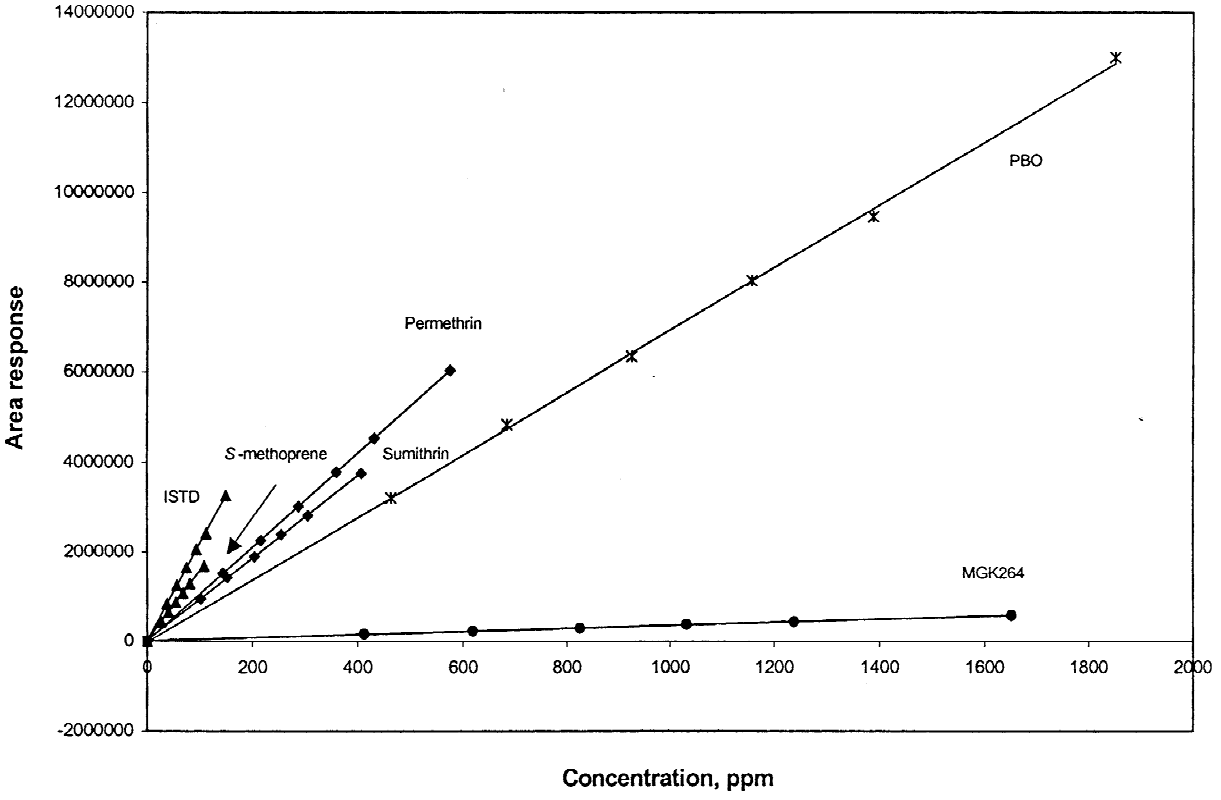


Fig. 4. Plots of linearity of chromatographic responses for (*S*)-methoprene, sumithrin, permethrin, PBO, and MGK264 in pesticide placebo sample.

PBO, MGK264, and internal standard in the placebo sample.

3.2.3. Accuracy

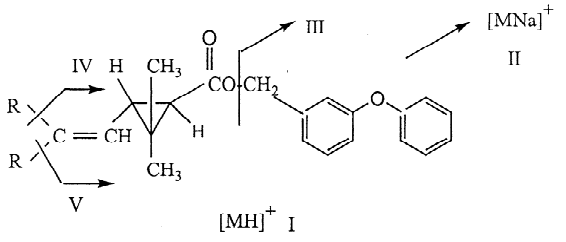
The accuracy of the proposed method was determined by recovery experiments. Known amounts of each active ingredient standard were spiked in the placebo samples. The percentage of recovery for each component was calculated based on the difference between experimental results and calculated results as shown in following equation.

$$\text{Recovery (\%)} = \text{component measured}/(\text{placebo sample} + \text{component added}) \times 100$$

Spiked recoveries were in the range of 93.44–104.36% with mean recoveries of 94.6–103.3%, indicating that the method has sufficient accuracy for the determination for the active ingredients in this pesticide formulation.

3.2.4. Method precision

The present method was applied to determine the amounts of each active ingredient in pesticide formulations. Six samples were prepared for the precision test. The samples were run with three independently prepared standards in the presence of internal standard. For each sample, three consecutive injections were carried out. The precision results on quantification of each component are summarized in Table 3. The relative standard deviations (RSDs) of the six samples were in the range of 0.8% to 1.9%. The RSDs are within the limit of acceptance criterion.



	R	I	II	III	IV	V
Permethrin	Cl	391.1	413.0	182.9	319.1	355.0
Sumithrin	CH_3	351.1	373.0	183.1	-	-

Fig. 5. RPLC–ESI-MS fragmentation patterns for permethrin and sumithrin.

3.3. Chromatographic peak identification

The chromatographic peaks were identified according to the molecular masses obtained by LC–ESI-MS in the positive mode. The mass spectra of MGK264, PBO, and (*S*)-methoprene were confirmed by the previous publications [10,15]. LC–ESI-MS fragmentation patterns of permethrin and sumithrin are included in Fig. 5. Both compounds showed clear molecular ions (m/z 391 and 351) and base ions (m/z 182.9 and 183.1). The MH^+ ion was located at m/z 391.1 and exhibited the characteristic isotopic pattern corresponding to the presence of two chlorine atoms in the molecule. The ions observed at m/z 319

Table 3
Precision results on quantification of each component in pesticide formulation

Standard	Sample	*S*-Methoprene	Permethrin	Sumithrin	MGK264	PBO
1	Sample A	0.114	0.433	0.375	2.484	1.882
	Sample B	0.114	0.437	0.379	2.481	1.886
2	Sample C	0.112	0.421	0.377	2.471	1.883
	Sample D	0.111	0.418	0.375	2.471	1.881
3	Sample E	0.111	0.420	0.370	2.432	1.883
	Sample F	0.112	0.424	0.373	2.445	1.880
Average		0.112	0.426	0.375	2.464	1.880
SD		0.001	0.008	0.003	0.021	0.002
RSD (%)		0.893	1.878	0.800	0.852	0.106

and 355 correspond to the Permethrin fragments $[MH-Cl]^+$ and $[MH-2Cl]^+$, respectively. The formation of strong signals for sodium adducts for both compounds was due to using ESI positive [19].

4. Conclusions

An isocratic RP-HPLC method for the analysis of (*S*)-methoprene, sumithrin, permethrin, PBO, and MGK264 in a new pesticide formulation has successfully been developed. The method development emphasizes the usefulness of including column selection and mobile phase change in optimizing a complex separation. This approach provides satisfactory results in finding an adequate isocratic RP-HPLC separation for a complex mixture consisting of analytes of widely different polarities and chromatographic behavior. By selecting a RP-C_8 column in combination with a ternary mobile phase, the RP-HPLC separation can reduce runtime, improve resolution, increase peak height, and eliminate the need for gradient separation. The present method showed retention time reproducibility after two-year stability study. Furthermore, all the five active ingredients in the formulation could be separated and determined in less than 30 min.

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