Determination of rotenoids and piperonyl butoxide in water, sediments and piscicide formulations

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Rotenone is a naturally occurring insecticide and piscicide (fish poison) found in many leguminous plants. This paper describes high-performance liquid chromatography (HPLC) methods for the quantitative analysis of rotenone's principal biologically active components (rotenone, tephrosin, rotenolone, deguelin) and the synergist piperonyl butoxide (PBO) in various media. Compounds were separated on a C_{18} reversed phase column with an acetonitrile–0.025 M phosphoric acid mobile phase and detected by UV absorbance or fluorescence (PBO only). Solid phase extraction (SPE) was used in either coupled (on-line) mode with a C_{18} concentrator column or automated off-line mode using Empore C_{18} disks. The on-line extraction efficiency was improved significantly by adding small amounts of methanol to water. Method detection limits (MDLs) for rotenoids and PBO in reagent water were 0.3 and 2 μg L^{-1}, respectively, with optimal recoveries ranging from 90% to 99%. Aquatic sediments were extracted with methanol and the extracts were diluted in water prior to analysis by coupled SPE-HPLC. In wet sediments, detection limits were approximately 20–100 μg kg^{-1} with recoveries of 71% to 87%. Sonication in dimethyl sulfoxide (DMSO) followed by dilution in acetonitrile and filtration allowed determination of the active ingredients in powdered rotenone formulations. Details of sample preparation, cartridge column cleanup and analyte confirmation are provided.

Introduction

Rotenone is a naturally occurring pesticide obtained from leguminous plants including *Lonchocarpus*, *Derris* and other species. Rotenone extracts have been used as piscicides and insecticides for centuries. The principal biologically active components of *Lonchocarpus* or cubé resin include the compound rotenone and the related rotenoid, deguelin, in which there is a six-membered E ring.[1] Rotenone has a specific conformation at the B/C ring fusion, but racemizes in alkaline media. Substitution at the 12a position with a hydroxyl group yields two additional naturally occurring rotenoids, rotenolone and tephrosin. The 12 position also undergoes facile isomerization and oxygenation in the environment,[2] and rotenolone and related compounds are rotenone transformation products in natural water. The principal rotenoids are shown in Fig. 1.

Rotenone is a metabolic poison that inhibits mitochondrial respiration. Rotenone toxicity is highly species specific with exceptional toxicity in gill breathing organisms.[1] Rotenoids are detoxified by cytochrome P-450 mixed function oxidases, enzymes inhibited by the aromatic aliphatic polyether piperonyl butoxide (PBO) (Fig. 2). As such, PBO is a rotenone synergist, and commercial piscicide formulations containing both materials have increased potency and residual activity.

Analytical methods for rotenone rely primarily on high-performance liquid chromatography (HPLC) because of rotenone's polarity and high molecular weight. In Bushway's HPLC method, 200 μL water samples are injected directly onto a reversed phase column giving a linear response between 15 and 300 μg L^{-1} for rotenone monitored at 210 nm.[3] Dawson and coworkers used solid phase extraction (SPE) on octadecylsilane cartridges prior to HPLC analysis, with absorbance detection at $\lambda_{max} = 295$ nm, to achieve detection limits in the range 5–20 μg L^{-1}.[4] HPLC has also been used to resolve epimers of rotenone and rotenolone[5] and PBO has been determined by HPLC[6] and electrospray HPLC-MS.[7]

SPE is popular because of reduced solvent consumption relative to liquid–liquid extraction (LLE). Instead of passing water samples by gravity through a sorbent cartridge, SPE can be carried out using liquid chromatography pumps and a concentrator column filled with HPLC column packing. This technique is referred to as on-line or coupled SPE. At the start of the analytical separation, a multiport valve is actuated and the sorbed sample is backflushed onto the analytical column. Coupled SPE-HPLC is used increasingly in drinking water laboratories for the determination of phenoxy herbicides and other compounds.

The purpose of this research was to further the development of HPLC methods for rotenone. We investigated coupled SPE-HPLC with absorbance, diode array and fluorescence detectors. The target compounds of interest included the major biologically active rotenoids as well as PBO. In addition, coupled SPE-HPLC was adapted for the determination of compounds in sediments and formulations.

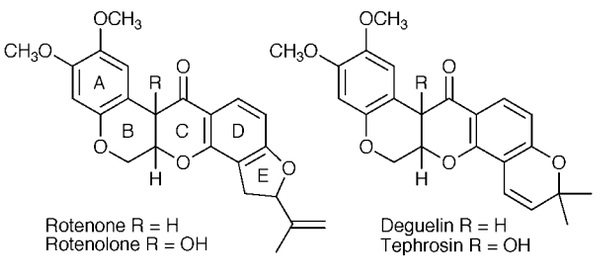

Fig. 1 Principal biologically active rotenoids in plant extracts.

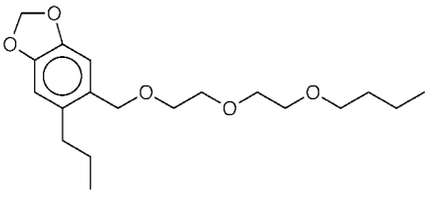

Fig. 2 Piperonyl butoxide synergist.

Methods and materials

Chemicals and supplies

Rotenone (97%) and PBO (technical grade, ~90% purity) were obtained from Aldrich (Milwaukee, WI, USA) and were used as received. Samples of rotenolone, tephrosin and deguelin were provided by N. Fang and J. Casida (University of California, Berkeley) and were isolated from cubé resin.[8] Nylon (#2934), polypropylene (#655606) and PTFE (#2384) 25 mm 0.45 μm syringe filters were obtained from Alltech (Deerfield, IL, USA). SEP-PAK C_{18} cartridges (#51915) were obtained from Waters (Milford, MA, USA). AgrEvo (Montvale, NJ, USA) kindly provided commercial rotenone formulations including powdered cubé root and Nusyn-Noxfish Fish Tox.

Standard solutions

Primary standards (2.0 mg mL^{-1}) were prepared in methanol and secondary and working calibration standards were prepared by serial dilution in acetonitrile. Standards stored at 4 °C in amber glassware were stable for many months—PBO was unchanged and <10% of rotenone was oxidized to rotenolone after 8 months. Rotenone solutions were stored under these conditions to minimize photodecomposition and oxidative breakdown.

Instruments

A gradient HPLC system with Isco Model 2300 pumps and an Isco Model 2301 gradient controller (Lincoln, NE, USA) was used. Samples were introduced with a loop injector or a concentrator column mounted on an electrically actuated, Valco C6W six-port valve (Cincinnati, OH, USA). The concentrator column was a Brownlee RP-18 Spheri-5 30 × 4.6 mm C_{18} cartridge (Alltech, Deerfield, IL, USA). The analytical column was a Supelco 25 cm × 4.6 mm 5 μm C_{18} Supelcosil LC-18 (Bellefonte, PA, USA). Supelco precolumns (2 cm) packed with either 5 μm C_{18} or 5 μm C_8 were used interchangeably without a noticeable difference in the separation. A Waters Model 490E variable wavelength UV/visible absorbance detector (Millipore, Milford, MA, USA) monitoring at 295 and 210 nm was used. Data acquisition and processing were carried out with a Dynamax MacIntegrator I (Varian, Walnut Creek, CA, USA). Some method development studies using diode array and fluorescence detectors were conducted with a Hewlett Packard 1100 HPLC system equipped with a quaternary pump, vacuum degasser and an autosampler (Wilmington, DE, USA).

HPLC separation

Optimal resolution of the target compounds required a non-linear (power of *n*) gradient with an initial concentration of 10% acetonitrile (ACN)–90% 0.025 M phosphoric acid and a final concentration of 70% ACN. This gradient rapidly increased the ACN fraction initially and improved the resolution of PBO from other formulation components that elute near the end of the chromatogram. The low initial ACN concentration is similar in solvent strength to the water sample concentrated with a maximum methanol concentration of 20% (v/v). The total mobile phase flow rate was 1.5 mL min^{-1}. For the Isco chromatograph, mobile phases were degassed under vacuum before use.

Concentrator column

A concentrator column was used for routine sample introduction. Water samples were transferred to a 50 mL graduated cylinder and acidified with four drops of 6 M hydrochloric acid. For water analysis, 20 mL samples were processed, while 20 or 40 mL sediment extract solutions were pumped through the concentrator at 4 mL min^{-1}. Surface water and sediment extracts were filtered through nylon or Teflon filter cartridges, respectively. For highest recoveries, water sample filters were rinsed with 5 mL of methanol and the methanol rinse was combined with the aqueous filtrate.

Calibration

The chromatograph was calibrated using either external standards in ACN introduced from a 10 or 25 μL loop or procedural standards prepared in water or aqueous methanol. Procedural standards were introduced *via* the concentrator column and gave better quantitative accuracy. Calculations were based on the peak height.

Off-line, automated solid phase extraction

In a limited comparative study, automated SPE was examined as an alternative to coupled SPE-HPLC. Water samples were extracted using 47 mm C_{18} 3M Empore disks (St. Paul, MN, USA) and a Tekmar Autotrace SPE workstation (Cincinnati, OH, USA). Water samples were processed in as little as 5 min with the disks and automation allowed unattended extraction of six samples with the apparatus. The preparation of disks, processing of samples and elution of analytes followed US Environmental Protection Agency (USEPA) Method 525.2 (Rev. 2.1), a GC-MS procedure for the analysis of semivolatiles in drinking water.[9] Briefly, 1 L water samples were treated with sodium sulfite to destroy residual chlorine, adjusted to pH < 2 with HCl and combined with 5.0 mL of methanol. The disks were extracted with ethyl acetate (EtOAc) and EtOAc–methylene chloride, the combined extract was dried with sodium sulfate and the final extract was obtained in 1.0 mL of EtOAc. Method 525.2 surrogates and internal standards, including five deuterated polycyclic aromatic hydrocarbons, were added to water at a concentration of 10 μg L^{-1}. These surrogates and internal standards were not needed, but were investigated as possible interferences in cases where the extract was to be analyzed by both GC-MS and HPLC. Method blanks, reagent water spiked with the four rotenoids (2.5 or 25 μg L^{-1}) and PBO (7.5 or 75 μg L^{-1}) and surface water samples from a rotenone-treated lake were analyzed. EtOAc extracts were injected directly onto the column with a 25 μL loop.

Determination of PBO by fluorescence detector

As an alternative to UV absorbance, detection analysis of PBO by a fluorescence detector (FLD) was examined in a limited comparative study. Large water samples (1.5 mL) were injected directly using the conventional 100 μL analytical head, a multidraw kit and a 1.4 mL extended seat capillary. The excitation and emissions wavelengths were 295 and 335 (optimal sensitivity) or 410 (optimal linearity) nm, respectively. The column used was a 10 cm × 2.1 mm C_{18} column with a linear ACN–water gradient.

Analysis of sediments

A sediment microextraction method was developed by modification of Dawson and Allen's sediment extraction procedure.[10] Any water present was poured off and the sediment was well mixed with a spatula before taking a subsample. Wet sediment (5 g) was transferred to a 50 mL Teflon centrifuge tube. Methanol (10 mL) was added and the sample was shaken vigorously for 3 min prior to low speed centrifugation (~1500 rpm) for 2 min. The methanol supernatant was filtered with a Teflon syringe filter and the filtrate was collected in a 50 mL volumetric flask. For direct analysis (no cleanup), the sample was adjusted to 50 mL with reagent water and acidified as before. The sediment extract at this point

was analyzed like a conventional water sample by concentrating 20 (or 40) mL on the concentrator column. A portion of the sediment sample was weighed before and after drying overnight at 100 °C to determine the dry weight.

C_{18} cartridge cleanup

Polar coextractives can be removed from sediment extracts with a C_{18} SEP-PAK cartridge cleanup. The methanol extract (10 mL) was combined with one volume of reagent water and then transferred to a washed C_{18} cartridge. The cartridge was prepared by rinsing first with 10 mL of methanol followed by 5 mL of reagent water. After loading the sample, the cartridge was washed with water and eluted with 10 mL of methanol which was combined with water for concentration as above.

Formulation analysis

Powdered rotenone (Pro-Noxfish Dust) was sonicated in dimethyl sulfoxide (DMSO) (10 mg mL^{-1}) and diluted in ACN. The 0.1 mg mL^{-1} ACN suspension was filtered to remove particulate matter and the filtrate was injected onto the chromatograph column using the loop injector.

Results and discussion

Chromatography of standards

On a C_{18} column, the elution order is rotenolone < tephrosin < rotenone < deguelin < PBO. Rotenolone and tephrosin were resolved using the power of *n* gradient which provided optimal resolution of PBO from other formulation components (Fig. 3). Dehydrorotenone and rotenonone, rotenone transformation products that elute after rotenone on reversed phase columns,[11] were not analyzed in the current study. Retention times for rotenone and PBO using various mobile phases are summarized in Table 1.

The chromatography of the target compounds was not affected by substituting water for dilute phosphoric acid in the mobile phase. The chromatogram shown in Fig. 3 is from a loop injection, but the peak shape, chromatographic efficiency and resolution were unchanged when samples were introduced *via* the concentrator column.

Solid phase extraction efficiency

The C_{18} concentrator column had extraction efficiencies between 40% and 53% for the rotenoids and 43% for PBO. Samples were acidified as recommended by Dawson and Allen,[10] although pH adjustment did not change the recoveries in coupled SPE. The extraction efficiency, however, was substantially improved by adding small amounts of methanol to the water sample prior to extraction. Adding only 2% methanol (v/v), for example, increased the recovery of rotenolone from 53% to 71%. The improvement in PBO recoveries was most pronounced, ~60%. The optimal amount of added methanol is ~10% where recoveries are uniformly high (69–74%) (Fig. 4). Even at 20% added methanol the chromatographic peaks are sharp and the resolution of the sample components is not degraded.

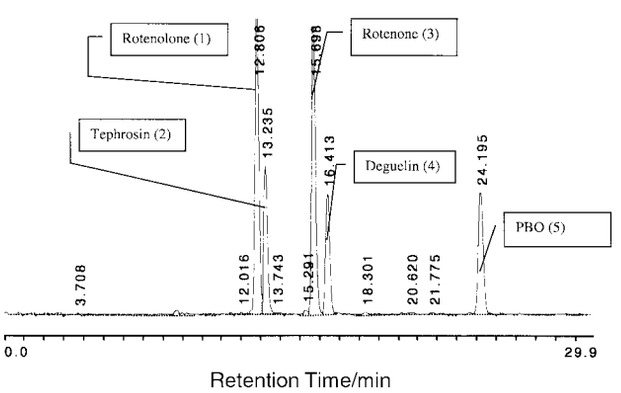

Fig. 3 Standard chromatogram.

Calibration

Coupled SPE-HPLC analysis was very sensitive with an S/N ratio for a 20 mL 1 μg L^{-1} rotenone solution of ~5. The detector response was approximately linear between 1 and 50 μg L^{-1} (Fig. 5) with a 12% RSD between response factors over this range. The absolute response for rotenone was greater at 210 nm, but there was more baseline and chemical noise at this wavelength. PBO had approximately one-fifth the response of rotenone at 295 nm, but also was linear between 10 and 50 μg L^{-1} (13% response factor RSD). Instrument detection limits (S/N ≅ 3) for rotenone and PBO (20 mL sample) were in the range 0.5–2 μg L^{-1} for rotenone (295 nm) and 2.5–5 μg L^{-1} for PBO (210 nm).

While the coupled SPE-HPLC system is generally linear, deviations from linearity can account for method bias particularly when the instrument is calibrated over a wide concentration range. Response factors increase at decreasing concentrations (Table 2) and, if the mean response factor is used, a positive bias of as much as 15–30% is anticipated at low concentrations. Better accuracy is achieved over a narrower concentration range, *e.g.* 2–20 μg L^{-1}. Quantification based on peak height also improves accuracy at low analyte concentrations.

Syringe filters and recoveries

Filtration is necessary for the analysis of surface water and sediment extracts.With some filter cartridges, analyte recovery is reduced due to sorption on trapped particles or the filter components. Rotenone was particularly susceptible to such losses, which varied greatly with filter type and were as high as 40% using Acrodise GHP cartridges. The contaminated cartridges cannot be reused because of carryover, a problem encountered even after thoroughly rinsing the cartridges with reagent water.

Recoveries using the polypropylene filters ranged from 51% to 79% (Table 3). Rinsing the filters with 5 mL of methanol reduced losses to between 1% and 10%. Tabulated recoveries are relative to the corresponding standard (*e.g.* with or without added methanol). Rinsing with solvent also extracted UV-absorbing contaminants from the filter cartridges, especially polypropylene filters, although none of the coextracted materials coeluted with the compounds of interest. Nylon filters imparted less contamination when filtering water and

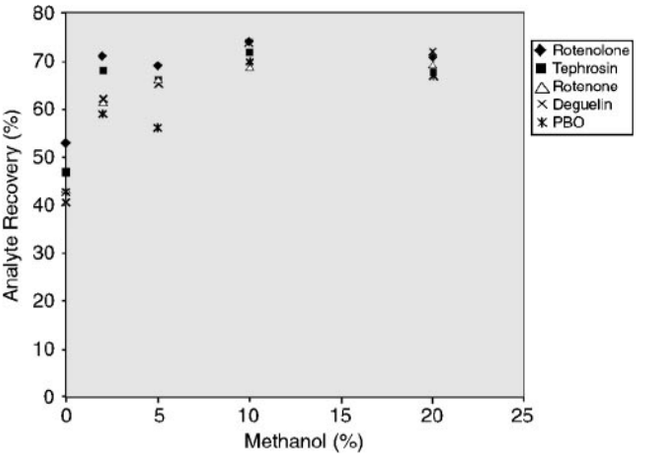

Fig. 4 Added methanol and on-line solid phase extraction efficiency.

Table 1 Typical retention times for rotenone and PBO

Mobile phase	Retention times/min	
	Rotenone	PBO
Isocratic 40% CAN–60% water	6.4	16.0
Isocratic 66% CAN–34% water	4.8	10.4
Linear gradient: 10–90% ACN (balance 0.025 M phosphoric acid) in 30 min	22.1 ± 0.02	26.9 ± 0.05
Non-linear gradient: profile 8, 10–65% ACN (balance 0.025 M phosphoric acid) in 30 min	13.7	24.1
Non-linear gradient: profile 7, 10–70% ACN (balance 0.025 M phosphoric acid) in 30 min	15.1	24.5

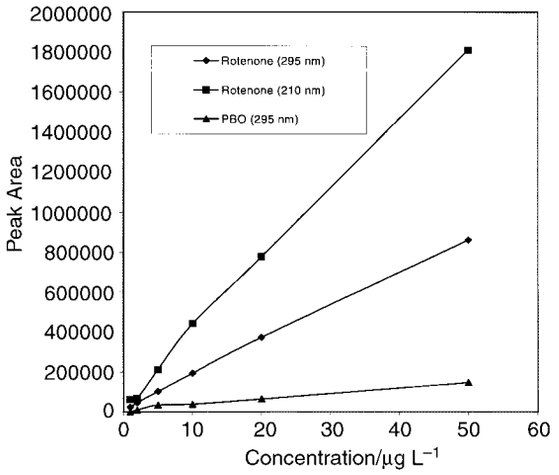

Fig. 5 Calibration curves for rotenone and PBO.

PTFE filters (used for the filtration of organic solvents) were virtually free of detectable contamination.

Method detection limits

For drinking water compliance monitoring in the USA, detection limits are based on the method detection limit (MDL).[12] MDLs were determined for two slightly different procedures (with and without filtration) using the coupled SPE-HPLC method. The spike levels were set based on the instrument detection limits and recoveries, and MDLs were determined both on a single day and over a 3 day period. For consistency, the chromatograms were each integrated with a computerized data system.

MDLs are affected by spike levels, minor changes in methodology and many other variables.[13] Rotenone's MDL is also strongly concentration dependent—at the lowest experimental spike level, 1 μg L^{-1}, MDLs of 0.2 μg L^{-1} (1 day) and 0.3 μg L^{-1} (3 days) were determined (Table 4). The rotenone MDL was 10-fold higher when quantification was based on peak areas, although this may vary with the data system used. A higher spike level, 2 μg L^{-1}, was used when filtering samples to compensate for the reduced recoveries (Table 5); under these conditions, experimental MDLs for each of the rotenoids were between 0.7 and 1.2 μg L^{-1}. In the 2 μg L^{-1} experiment, not all of the rotenoids were detected in each of the replicates. At the MDL concentration, the probability of false negatives increases while false positives are unlikely. Because MDLs are determined in laboratory reagent water, they may not reflect laboratory performance in actual samples, and it is common to set laboratory reporting limits at a multiple of the MDL (*e.g.* two to five times) to improve data quality. The region of uncertain quantification is between two and ten times the MDL. The calculated MDL for PBO was 2 μg L^{-1} in either experiment.

Table 2 Rotenone calibration and bias in coupled SPE-HPLC[a]

Rotenone/ μg L^{-1}	Peak area		Peak height	
	Response factor	Bias (%)[b]	Response factor	Bias (%)[b]
1	23400	15	3140	30
2	22800	12	2500	1
5	20600	1	2460	0
10	19400	−5	2360	−4
20	18700	−8	2250	−8
50	17200	−15	2020	−18

[a]20 mL sample volume, UV absorbance at 295 nm. [b]Anticipated bias when using the mean response factor for the entire calibration range.

Table 3 Recovery of analytes sorbed in cartridge filters

Treatment	Analyte recovery *vs.* control (%)				
	Rotenolone	Tephrosin	Rotenone	Deguelin	PBO
Filter	79	72	64	51	52
Filter/rinse	98	93	99	90	92

Confirmation techniques

Confirmation is important due to the low specificity of the absorbance detector. Monitoring the absorbance at two wavelengths is useful for this purpose and readily distinguishes analytes from interferences in high dissolved organic carbon (DOC) waters or sediment extracts. The rotenone/rotenolone chromophore has an absorbance maximum at 295 nm, while the tephrosin/deguelin chromophore absorbs maximally at 270 nm. Absorbance ratios at these wavelengths are distinctive (Table 6). PBO has maxima at 238 and 290 nm.

Sediment analysis

Sediment samples from a northern California lake were analyzed without supplemental C_{18} cartridge cleanup. The sediment chromatogram was complex and had peaks that corresponded to the analytes, but none exceeded reporting limits or had the appropriate absorbance ratio. This sediment

Table 4 Coupled SPE-HPLC MDL data

	Rotenone/μg L^{-1} [a]	Piperonyl butoxide/μg L^{-1} [a]
Day 1	1.6	3.5
	1.4	3.4
Day 2	1.6	3.6
	1.5	3.7
Day 3	1.5	3.6
	1.4	4.9
	1.5	3.9
	1.4	4.8
	1.3	3.8
	1.3	4.7
	1.4	5.2
Overall mean	1.45 (145% recovery)	4.09 (136% recovery)
One day MDL	0.18	1.6
Three day MDL	0.28	2.0

[a]Formulation in reagent water containing 1 μg L^{-1} rotenone and 3 μg L^{-1} PBO. Rotenone (295 nm) and PBO (210 nm) by peak height.

Table 5 Coupled SPE-HPLC MDL data (filtered samples)

	Concentration/μg L^{-1} [a]				
Replicate	Rotenolone	Tephrosin	Rotenone	Deguelin	PBO
#1	1.9	1.8	1.4	0.57	8.9
#2	1.4	1.5	0.82	0.70	8.0
#3	2.0	1.5	1.2	0.90	8.1
#4	2.1	1.7	1.2	1.3	8.0
#5	1.6	1.4	1.2	0.99	8.0
#6	1.1	ND	ND	ND	9.3
#7	1.9	1.0	0.89	0.29	7.9
Mean	1.7	1.5	1.1	0.79	8.3
Recovery (%)	86	74	56	40	140
MDL/μg L^{-1}	1.2	0.92	0.72	1.2	1.7

[a]Formulation: 2 μg L^{-1} (rotenoids) and 6 μg L^{-1} PBO in laboratory reagent water; all compounds detected at 295 nm, samples filtered with polypropylene filter; filters not solvent rinsed.

sample was spiked with the analytes at two levels: a low level spike with 100 μg kg^{-1} of the rotenoids and 300 μg kg^{-1} PBO and a high level spike with twice these concentrations. The average recoveries for the two spike levels were: rotenolone (73%), tephrosin (77%), rotenone (71%), deguelin (87%) and PBO (75%). In each case, the absorbance ratios were in good agreement with the standards. Lake sediment spiked at the low concentration is shown in Fig. 6(a). Estimated detection limits for a 20 mL aqueous methanol sample, equivalent to 2 g of wet sediment, are ~20 μg kg^{-1} for the rotenoids and 100 μg kg^{-1} for PBO.

The ratio of methanol to wet sediment is important for high recoveries. Large sediment samples contribute significant quantities of water and a sufficiently high methanol to water ratio is needed for efficient extraction. The sensitivity of the present microextraction coupled SPE method appears to be similar to the macroextraction procedure of Dawson and Allen[10] which uses solvent partitioning and silica gel column chromatography in the preparation of sediment samples.

Formulation analysis

Various organic solvents were tested to improve the dissolution of rotenone from a powder formulation (methanol, chloroform, isooctane, 95% ethanol and DMSO). Sonication in DMSO appeared to be most effective, but filtration after dilution in ACN was still necessary. The detection limits for active ingredients were in the range 1–2% on a weight basis. The dust analyzed contained rotenone (average 6.6 wt.%) and deguelin (average 5.7 wt.%) and trace quantities (<1%) of rotenolone and tephrosin [Fig. 6(b)]—each compound had the appropriate absorbance ratio. Other methods for the extraction of rotenone from derris and cubé powder are described in AOAC method 983.06.[14]

Off-line solid phase extraction

Off-line SPE with Empore disks provided an alternative to coupled SPE-HPLC. EtOAc extracts were analyzed by 25 μL loop injection with no deterioration of the peak shape. With the 1000-fold concentration factor, sensitivity was in the low ppb range, equivalent to coupled SPE with a 25 mL sample. Surrogates and internal standards (IS) are seen in the extract chromatogram [Fig. 7(a)], but they do not coelute with any of the target compounds except for a minor peak coeluting with tephrosin. At the low spike concentration, the recoveries were in the range 87–100% and recoveries of all five target compounds were in the 74–82% range at 25/75 ppb [Fig. 7(a)]. In moderately high DOC surface water, polar compounds eluted just after the solvent peak [Fig. 7(b)] with the signal returning to baseline before the earliest eluting target compound. Samples from a lake treated with rotenone contained 2 μg L^{-1} PBO [Fig. 7(b)]. The absorbance ratio

Table 6 Absorbance at different wavelengths

		Relative absorbance			Absorbance ratio[a]	
Compound	t_R/min	210 nm	270 nm	295 nm	A_{210}/A_{295}	A_{270}/A_{295}
Rotenolone	13.6	1000	79	446	2.24	0.18
Tephrosin	14.0	538	427	110	4.89	3.88
Rotenone	16.3	737	67	329	2.24	0.20
Deguelin	17.0	428	370	106	4.04	3.49
PBO	24.4	322	46	130	4.5[b]	0.35[b]

[a]From diode array detector except where noted. [b]From UV/visible absorption spectrum of ~90% technical material in methanol or ACN.

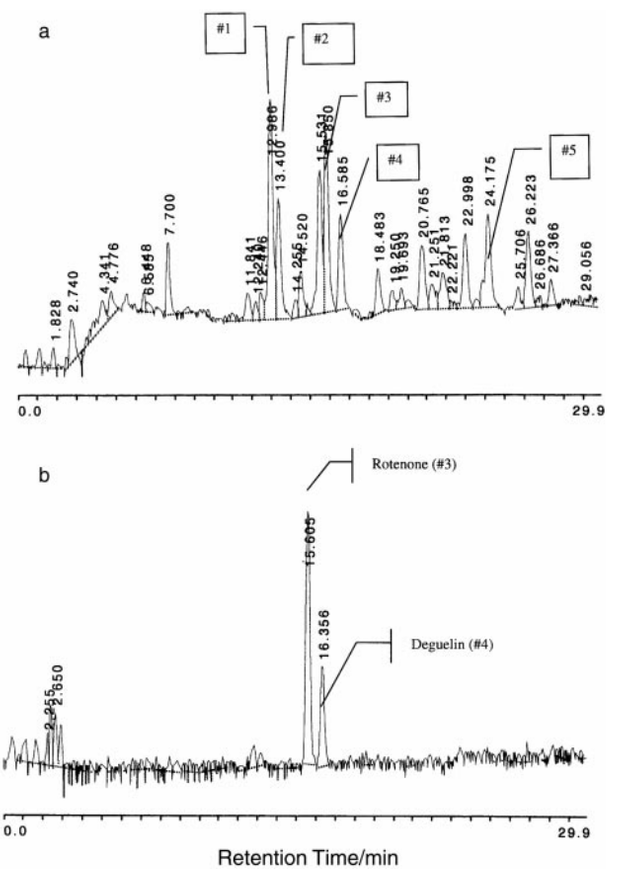

Fig. 6 (a) Lake bottom sediment spiked with rotenoids (100 μg kg^{-1}) and PBO (300 μg kg^{-1}). (b) Rotenone dust formulation containing 6.1 wt.% rotenone and 5.8 wt.% deguelin.

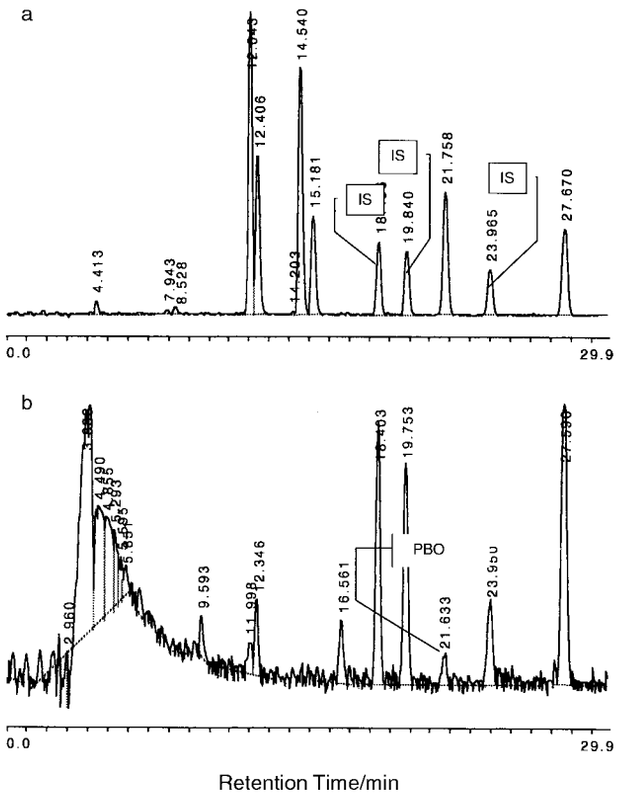

Fig. 7 (a) Chromatogram from automated off-line SPE analysis of reagent water spiked with rotenoids (25 μg L^{-1}) and PBO (75 μg L^{-1}). (b) Rotenone-treated surface water with trace PBO residue (~2 μg L^{-1}).

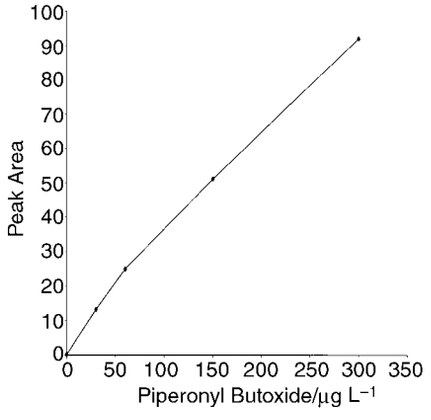

Fig. 8 Direct fluorescence-HPLC determination of PBO in water (295 nm excitation/410 nm emission).

(A_{210}/A_{295}) was equivalent to the PBO standard and the result was also confirmed by both coupled SPE-HPLC and GC-MS.

Determination of PBO by fluorescence detector

All of the above data were based on absorbance detection, but PBO also fluoresces allowing an alternative detection mode. A further modification of the HPLC analysis involved direct injection of large (1.5 mL) water samples directly onto a reversed phase column. The linear mobile phase gradient was adjusted giving retention times of 13.42, 13.62, 14.49, 14.74 and 16.75 min for the analytes in the same elution order and with rotenoids determined solely by diode array detection and PBO detected by FLD as well. With excitation of PBO's 295 nm absorption band, the optimal emission was at 335 nm. Monitoring the emission at 410 nm, however, provided a greater linear dynamic range for the standards analyzed which varied in concentration between 30 and 300 μg L^{-1} (Fig. 8). The detector response was 50 times greater at the maximal emission wavelength, indicating that direct FLD-HPLC analysis is potentially very sensitive.

Conclusions

Coupled SPE-HPLC is well suited to the determination of rotenoids and PBO in drinking water and surface water. Piscicide residues in sediments can also be accommodated by this technique after a simple microextraction procedure followed by dilution of the extract in water. Filtering samples with plastic cartridge filters can result in substantial losses of the analytes and solvent rinsing of the filters is necessary for quantitative recovery. The addition of small quantities of methanol (10% v/v), which may come from cartridge filter rinsates or sediment extracts, improves the recovery of residues in coupled SPE-HPLC analysis. The specificity of the HPLC method is improved through monitoring multiple wavelengths or diode array spectra. Further study is needed to evaluate FLD-HPLC for the determination of PBO in surface water and other matrices, although the available data suggest that this technique may be very sensitive.

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