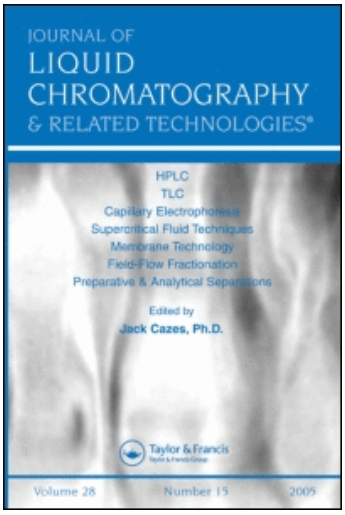

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HPLC AND GC/MS OF METRIBUZIN AND ITS DEGRADATION PRODUCTS FROM ALGINATE-LINSEED OIL CONTROLLED RELEASE FORMULATIONS

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ABSTRACT

In the preparation of alginate-clay-linseed oil controlled release formulations of metribuzin and the evaluation of their release rates, degradation of the metribuzin was observed. The degradation products were separated by HPLC and identified by GC/MS as diketometribuzin (DK), deaminated metribuzin (DA), and deaminated diketometribuzin (DADK). The HPLC method developed afforded baseline separation without the use of acid or buffers in the mobile phase. These same products are the major metabolites of metribuzin in plants and soil. The production of DK was directly proportional to the amount of linseed oil in the formulation whereas DA appears to be inversely proportional to linseed oil concentration.

INTRODUCTION

Groundwater has become contaminated by man's activities because it inadvertently serves as a sink for wastes from accidental spills, runoff of agricultural and domestic fertilizers, pesticides, septic tank leaks, and numerous other sources of pollution. Pesticides do not contribute to groundwater pollution on a magnitude similar to landfills and underground storage tanks. However, large quantities of pesticides are used

extensively throughout the United States and several have been identified as sources of groundwater contamination [1,2]. Point source contamination from spills, excessive application and improper disposal intensify the problem. Pesticides such as metribuzin, atrazine, alachlor, aldicarb, and carbofuran, which are known to have some degree of persistence in the environment, are among those implicated as groundwater contaminants.

Agrochemicals find their way into the groundwater through runoff, leaching, volatilization, and improper application. Application as controlled release formulations (CRF) would have a significant positive impact on both volatilization and application [3], and may reduce leaching [4,5].

Our current research involves the use of modified alginate formulations for the production of CRF. Other researchers have utilized alginates in combination with various additives to prepare CRF of herbicides [6-7]. In studies with 2,4-D and dichlobenil, Connick concluded that the release rate from alginate beads was directly related to the water solubility of the herbicide [7]. The use of fillers such as clay caused some alteration of the release rates. The authors incorporated adsorbents into an alginate-clay formulation to modify the release characteristics of the herbicide metribuzin and found that charcoal reduced the rate of release [8]. They also incorporated linseed oil into an alginate-clay formulation to modify the release characteristics of the herbicides metribuzin, atrazine, and alachlor [9]. The initial data indicated that addition of linseed oil to the formulation had the desired effect of moderating the release of the herbicides. However, in the case of metribuzin, there was evidence that degradation products were formed during the formulation process. To determine the effective active ingredient present (actual amount of metribuzin present in the formulation) and the conditions required to minimize the degradation of metribuzin, we examined the effects of varying the oil content of the formulation on the formation of these products. The degradation products from

metribuzin were separated by high performance liquid chromatography (HPLC) and identified by gas chromatography/mass spectrometry (GC/MS).

MATERIALS

Chemicals and Standards

Metribuzin [MBZ, 93-94% pure, 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one], deaminated metribuzin [DA, 99.6% pure, 6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one], diketometribuzin [DK, 98.7% pure, 4-amino-6-(1,1-dimethylethyl)-1,2,4-triazin-3,5(2H,4H)-dione], and deaminated diketo metribuzin [DADK, 84.7% pure, 6-(1,1-dimethylethyl-1,2,4-triazin-3,5(2H,4H)-dione] were provided by Mobay Corporation, Kansas City, MO [10]. Metribuzin was recrystallized as previously described until no significant impurities were detectable by HPLC [8]. DA, DK, & DADK were used as provided without further purification.

Sodium alginate, Kelgin MV, was obtained from Kelco, Division of Merck and Company, San Diego, CA and Kaolin clay from Thiele Kaolin Company, Wrens, GA.

Raw and boiled linseed oil (produced by T&R Chemicals, Inc., Clint, Texas) was purchased at local hardware stores, and Tween 20 was purchased from Atlas, ICI America, Inc., Wilmington, DE.

Deionized water purified with a Water-I purification system from Barnstead (Division of Sybron Corporation, Boston, MA) was used throughout the study.

Other chemicals were either HPLC grade or reagent grade.

METHODS

Formulation and Extraction Procedures

A typical preparation of alginate gel beads containing metribuzin comprised mixing the metribuzin (1.0 g), the linseed oil (0-10 g), and Tween 20 (1.0 g) together with a magnetic

stirrer for 15-20 minutes. The clay (10 g) and water (77-87 g) were added and the pH adjusted to 7.8 with 5% sodium hydroxide. Alginate was added (1.0 g) while stirring vigorously with an overhead stirrer. After one hour the pH was readjusted to pH 7.8 with NaOH and the slurry stirred for 10 minutes more. The uniform slurry was then added dropwise through Pasteur pipets into a dilute solution (0.25M) of $CaCl_2$ (about twice the weight of the slurry to be dropped) causing gelation of the alginate mixture. The amount of formulation added to the $CaCl_2$ was weighed, and the beads were allowed to harden for 2-5 minutes before removal of excess $CaCl_2$ solution by vacuum filtration through a coarse-frit Buchner funnel. The beads were allowed to stand in the funnel for 2-4 hours with occasional removal of water due to syneresis, then spread on aluminum foil to air-dry. Beads prepared in this manner contained 2-6% active ingredient (a.i.).

A typical controlled release study of metribuzin formulation in water was conducted by adding 2-4 g of metribuzin formulated beads to 1 l of purified water in a 1 l stoppered glass Erlenmeyer flask. The amount of beads, based on % a.i. in the formulation, was selected so that the theoretical maximum release of metribuzin from the formulation would not exceed 200 ppm. Water solubility of metribuzin is approximately 1200 ppm at ambient temperatures. A 3 ml aliquot of the aqueous solution was taken at prescribed intervals. Immediately prior to taking the sample, sample flasks were gently shaken by inversion of the flasks several times.

The formulation extract for GC/MS analysis was prepared by methylene chloride extraction of about 1 l of aqueous solution. This aqueous solution was the supernatant from extraction of 2-4 g of alginate-clay-linseed oil-metribuzin beads (2-6% a.i.) for 25 to 40 days. The supernatant was separated from the beads by decanting and was extracted twice with 50 ml methylene chloride (CH_2Cl_2). The extracts were combined and dried with anhydrous sodium sulfate, then evaporated to dryness under vacuum on a rotary evaporator to yield a yellowish oily residue. One ml of methanol was added to dissolve

the residue and the yellow solution filtered through glass wool (packed at the tip of a disposable pipet). Filtrate was concentrated if necessary.

HPLC Analysis

The high performance liquid chromatographic system consisted of a WISP 710B autosampler, a 6000A pump, a Lambda-Max 480 LC Spectrophotometer detector, set at 254 nm and absorbance attenuation of 1 auf (all from Waters Associates, Milford, MA), and a linear chart recorder model D5217-5 (Houston Instrument, Austin, TX). Data acquisition and peak integration were accomplished with a HP3357 Laboratory Automation System, LAS (Hewlett-Packard, Avondale, PA). A Waters Resolve C18, 15 cm long x 3.9 mm I.D., 5 micron particle size, stainless steel column was used. The mobile phase solvent was methanol: water (50:50, v/v), isocratic at a flow rate of 0.8 ml/min. The WISP was set at auto mode, 50 ul/injection, 15 min. run time, and 2 runs/sample.

Stock standards of MBZ, DA, DK, and DADK (10 mg/ml) were prepared in HPLC-grade methanol. Working standards, 1-200 ppm, were prepared from the dilution of stock standards with purified water.

Three ml samples, were filtered through 0.22 um Millex-GV filters (Millipore Corporation, Bedford, MA). Standards of metribuzin were run routinely as a control.

GC/MS Analysis

A Perkin-Elmer Sigma 300 GC/Finnigan MAT 800 Ion Trap Detector (ITD) was used for separation and identification of the metribuzin degradation products. The GC conditions used were: injector temp. 220°C, split ratio 20:1; oven temp. 200°C isothermal. Helium was used as carrier gas at a flow rate of 1.45 ml/min (linear velocity 30 cm/sec) at 100°C. The GC column was a Hewlett-Packard Ultra-2 (cross-linked 5% phenylmethyl silicone) capillary column, 50 m long x 0.32 mm I.D., 0.52 micron film thickness. The

transfer line temperature was set at 250°C. The ITD was operated on electron-impact (EI) mode with electron multiplier voltage at 1550 volts, and the mass was scanned from m/e 45-350 at 1 scan/sec. There was a 360 sec. filament/multiplier delay to avoid the solvent peak.

MBZ, DA, DK, DADK working standards, 400 ng/ul, were prepared from the dilution of 10 mg/ml stock standards (in methanol) with reagent grade methanol. To determine the retention time (RT) and to generate the mass spectra of these compounds, 400-800 ng were injected into GC/ITD (20-40 ng of compounds actually went into ITD due to 20:1 split in the injector). The formulation extracts, 1-2 ul, were run under the same conditions as the standards. The presence of MBZ, DA, DK, and DADK in the extracts was confirmed if their retention times and mass spectra closely matched those of the authentic compounds.

RESULTS AND DISCUSSION

HPLC Analysis

The degradation products of metribuzin -- deaminated metribuzin, deaminated diketometribuzin, and diketometribuzin -- have been reported by several researchers as metabolites in various crop plants and in soil [11-13]. Several researchers have reported HPLC methods for the separation of metribuzin and its metabolites [14-16] and we used these as guides to develop a suitable method for our substrate. In the method we developed, baseline separation and good resolution of all the peaks were obtained without resorting to the use of buffers or acid. A standard calibration curve for metribuzin was constructed and was linear over the range of 1-200 ppm. Concentrations of metribuzin released from the formulations were calculated, based on the standard calibration curve. To determine the retention time (RT) and response factor (RF = peak

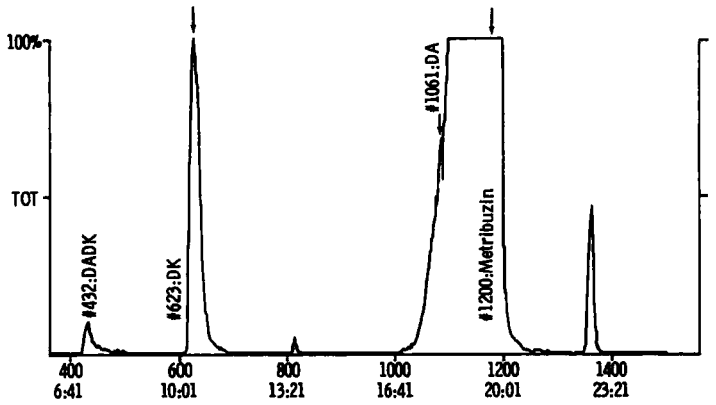

Figure 1 - HPLC chromatogram of metribuzin (3) and its degradation products [DK (1), DADK (2), AND DA (4)] from a typical alginate-clay-linseed oil formulation.

area counts/ppm) of DA, DK and DADK, working standards at the concentration of 100 ppm were run individually under the same conditions as for metribuzin. The relative retention times (RRT) of DA, DK, and DADK to MBZ were then established and used as the references for the identification of these compounds in formulation extracts. The relative response factor (RRF) was defined as the ratio of the response factor of a certain compound to the response factor of metribuzin. The RRF of DA, DK & DADK were used to calculate the concentrations of these compounds in formulation extracts. Under normal conditions (see Methods section), the elution order of these compounds were DK, DADK, MBZ, then DA; their RRT were DK:DADK:MBZ: DA=0.5867, 0.6966: 1.0000:l.0849; and their corrected (for purity) RRF were DK:DADK:MBZ:DA=3.66:4.51:1.00:7.00 at 254 nm. A typical HPLC chromatogram of a formulation extract is depicted in Figure 1, in which peaks #1,2,3, and 4 were identified as DK, DADK, MBZ, and DA with RTs of 4.70, 5.58, 8.01, 8.69 min., respectively. Extracts of a formulation containing all ingredients

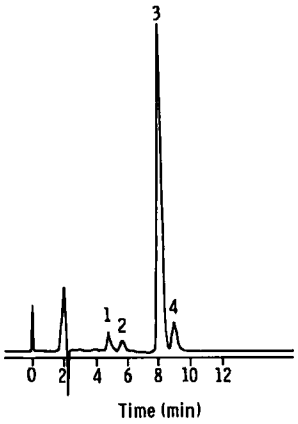

Figure 2 - GC/MS total ion chromatogram of the extract of an alginate-clay-linseed oil formulation containing metribuzin.

except metribuzin showed no peaks associated with metribuzin or its degradation products.

GC/MS Analysis

To confirm that the observed HPLC peaks were indeed degradation products of metribuzin, water extracts of the formulations were extracted with methylene chloride. The methylene chloride extracts were then run under the same conditions as the standards. They were identified and confirmed by the comparison of GC retention times and the matched mass spectra with the standards. A total ion chromatogram of a formulation extract is depicted in Fig. 2.

The mass spectrum of metribuzin was found in the NBS-EPA library of ITD data system, but not the mass spectra of DA, DK & DADK. When standard metribuzin was run by GC/ITD, the mass spectrum obtained was different from that in the library resulting in

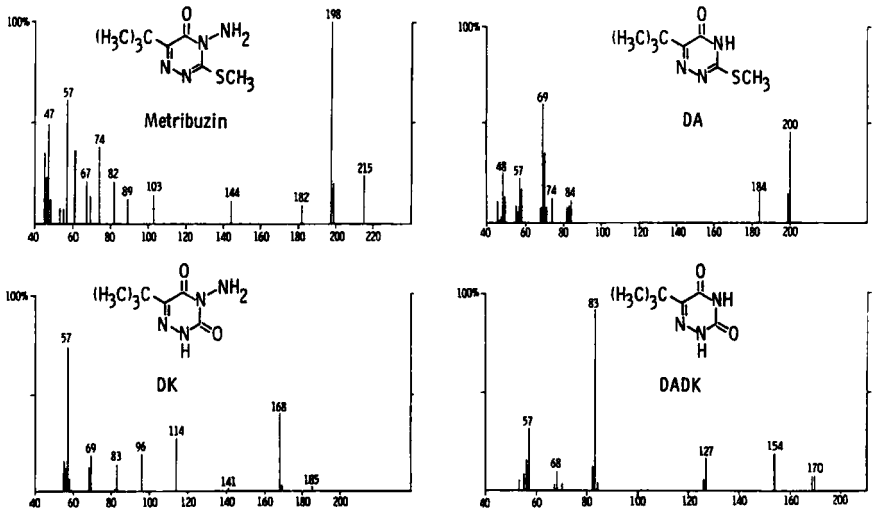

Figure 3 - GC/ITD mass spectra of metribuzin and its degradation products.

low matched numbers. It became obvious that we needed to generate our own mass spectra of these compounds on our ITD to reach a definitive confirmation. Standard DA, DK, DADK, and MBZ were injected individually onto GC/ITD to obtain their mass spectra and to determine their retention times (actually scan numbers). The elution order of these compounds on the GC column was different from the HPLC column and followed the order: DADK, DK, DA & MBZ. Under the described conditions (see GC/MS analysis in Methods section), the scan numbers (i.e., RT in seconds) of these standards were: DADK-421, DK-611, DA-982, and MBZ-1084. Protonated molecular ions, $(M+1)^+$, were observed in all four compounds, DA, DK, DADK & MBZ. This commonly occurs for polar compounds generated by an ITD [17]. Their mass spectra and structures are shown in Figure 3.

Table 1
Concentrations (ppm) of Metribuzin Metabolites Found in Water Extracts of Alginate Linseed Oil Formulations

		Time (hours)					
Compound*	Sample**	1	8	48	144		
DK	A	0.69	2.00	4.76	5.54		
	B	0.90	2.33	4.80	7.22		10.01
		0.75	1.90	3.84	5.88		
		0.40	0.99	2.09	3.33		
		0	0.27	0.68	1.02		
	F						
DADK	A	0	0.54	0.77	0.83	0.89	1.04
	B	0.20	0.54	0.76	0.81	0.89	1.05
	C	0.19	0.56	0.73	0.80	0.84	0.96
	D	0.22	0.60	0.75	0.79	0.86	0.95
	E	0.28	0.66	0.79	0.83	0.87	1.01
	F	0	0	0	0	0	0
DA	A	0.12	0.36	0.78	0.89	1.06	1.26
	B	0.17	0.42	0.92	0.98	1.12	1.34
		0.17	0.45	0.87	1.00		1.39
	D	0.20	--	0.87	1.04	1.16	1.49
	E	0.25	0.67	1.11	1.35	1.50	1.99
		0	0	0	0	0	0

* DA=diketometribuzin, DA=deaminated metribuzin, DADK=deaminated diketometribuzin.

Degradation of Metribuzin in Formulations

No significant conversion of metribuzin to DA, DK, DADK was found in metribuzin-alginate formulation extracts when linseed oil was absent from the formulations. However all three of these compounds were found in the extracts of metribuzin-alginate formulations containing linseed oil (see Table 1). The concentration of the total degradation products (ppm) extracted (744 hours) varied from 22% of the metribuzin (ppm) extracted for formulation A to only 3.5% for formulation E. This is calculated by adding up the total ppm of the degradation products for a formulation at 744 hrs and dividing by the ppm of metribuzin at 744 hrs (data not shown).

To determine whether the conversion of metribuzin was occurring after release from the formulation, pure metribuzin was dissolved in water: one sample in neutral solution (pH=6.7) and one in acidic solution (pH 5.4, adjusted with diluted HCl). The metribuzin solutions were analyzed periodically (up to one year) to determine the stability of metribuzin under these conditions which were similar to the controlled release tests. Results on the stability study (see Table 2) indicated that metribuzin hydrolyzed very slowly, producing small amounts of DA after 56 days in acidic solution and 70 days in neutral solution, only a trace of DK after 75 days in acidic solution and no measurable amounts of DADK during this time frame. Most investigators agree that the degradation pathway is stepwise with the initial formation of DA or DK and conversion of either to DADK [13]. DA has been reported as the major photodegradation product in aqueous solutions [18]. This study demonstrated that decomposition of metribuzin was faster in acidic medium than in neutral one. Our own results with the alginate formulation and discussions with personnel at Mobay Corporation indicated that metribuzin is very stable under slightly alkaline conditions. Some of our earlier work with metribuzin in alginate formulations without using a basification step resulted in extensive degradation of the

Table 2

Decomposition of Metribuzin in Water

DAYS		DK(ppm)		DADK(ppm)		DA(ppm)	
N	A	N	A	N	A	N	A
1	5	0	0	0	0	0	0
19	41	0	0	0	0	0	0
55	56	0	0	0	0	0	1.64
70	75	0	TRACE <0.3	0	0	1.42	2.18

N - standard metribuzin in water without pH adjustment, 114 ppm, pH 6.7.

A - same standard as in N adjusted to pH 5.4 with 5% HCl.

DA = diketometribuzin, DA = deaminated metribuzin, DADK = deaminated diketometribuzin.

herbicide. It was at this juncture in the research that we began routinely basifying all of our metribuzin CRF preparations.

From the controlled-release study of metribuzin-linseed oil formulations in water, the concentrations of DA, DK & DADK found in aqueous formulation extracts were considerably higher than those found in aqueous solutions of pure metribuzin. These results demonstrate that interaction of metribuzin with linseed oil during the formation of the alginate-linseed oil beads caused degradation of metribuzin to DA, DK, and DADK. The degree of degradation depended on the amount of linseed oil used for the formulation and acidity of formulation medium. In particular, the concentration of DK was directly related to the amount of oil in the formulation whereas DADK was present at about 1.0 ppm in all formulations containing linseed oil. DA was slightly dependent on the percent oil in the formulation increasing from 1.25 ppm to 1.99 ppm as the

concentration of oil decreased. Even after 744 hours there was no evidence of any of the metabolites in the water extracts of the alginate formulations which did not contain linseed oil. The results in Table 1 occurred despite adjustment of the formulation mixture to pH 7.6-7.8 to minimize degradation.

CONCLUSIONS

In the course of preparation and evaluation of release rates of controlled release formulations of metribuzin, it became evident that the use of linseed oil as an additive to the alginate-clay mixture to moderate the release of the herbicide caused the degradation of a portion of the metribuzin. The degradation products were separated by an HPLC method developed for this substrate which avoided the use of acid or buffers in the mobile phase. The products were tentatively identified as diketometribuzin, deaminated metribuzin, and deaminated diketometribuzin from retention time comparisons to authentic standards. Absolute identification was achieved by the use of GC/MS. The amount of degradation was directly proportional to the amount of linseed oil in the formulation and suggested that only the lower amounts of linseed oil (2-4 %) would be practical in a controlled release formulation.

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