Journal of Chromatography, 474 (1989) 301–316
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 448

COMPARISON OF OPEN-TUBULAR LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY AND DIRECT LIQUID INTRODUCTION LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY FOR THE ANALYSIS OF METRIBUZIN AND ITS METABOLITES IN PLANT TISSUE AND WATER SAMPLES

B. H. ESCOFFIER[a], C. E. PARKER*, T. C. MESTER[b] and J. S. M. DEWIT[c]
Laboratory of Molecular Biophysics, N.I.E.H.S., Research Triangle Park, NC 27709 (U.S.A.)
F. T. CORBIN
Crop Science Department, North Carolina State University, Raleigh, NC 27695 (U.S.A.)
J. W. JORGENSEN
Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 (U.S.A.)
and
K. B. TOMER
Laboratory of Molecular Biophysics, N.I.E.H.S., Research Triangle Park, NC 27709 (U.S.A.)

SUMMARY

Direct liquid introduction (DLI) and open-tubular liquid chromatography–mass spectrometry (OTLC–MS) have been evaluated for the analysis of metribuzin and its metabolites in water and soybean samples. To improve sensitivity and column performance, a column-switching procedure was developed for on-line analyte preconcentration and sample cleanup in DLI LC–MS. To reduce the amount of sample needed for OTLC–MS injection, a "dip" technique analogous to on-column injection in high-resolution gas chromatography (HRGC)–MS was used. Although OTLC was more sensitive than DLI for mixtures of pure standards, severe matrix effects were encountered when plant and water samples were analyzed by this technique. DLI LC–MS was more successful than OTLC–MS for environmental samples due to both the inherent loading capacity of the larger column and the use of the preconcentration/cleanup injection technique.

INTRODUCTION

Metribuzin (MZN) is a 1,2,4-triazine herbicide which has been widely used on foodcrops, including soybeans, tomatoes, and sugarcane. Differences in crop tolerance to this herbicide has led to studies of its metabolism. Three major metabolites are

[a] Present address: Groupement de Recherches de Lacq, D.A.A., 64170 Artix, France.
[b] Present address: Landis Associates, Placerville, CA 95667, U.S.A.
[c] Present address: Tennessee Eastman Co., Kingsport, TN 37662, U.S.A..

0021-9673/89/$03.50

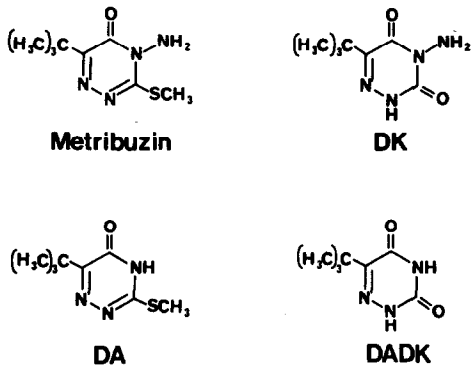

Fig. 1. Reported structures for metribuzin, DK, DA, and DADK.

presently known: deaminated metribuzin (DA); diketometribuzin (DK) and deaminated diketometribuzin (DADK)[1–4] (see Fig. 1). Metabolism of these compounds via glucoside and glutathione conjugation has also been studied[5,6]. Concern over this herbicide entering the food chain through the ingestion of residues on crops has led to its study in crops, food, milk and tobacco[7–10]. Another area of concern is the effect on subsequent crops through soil residues[11–13] and on the environment through runoff and groundwater contamination[14–23]. MZN has been placed on the U.S. Environmental Protection Agency (E.P.A.) list of cancer-suspect agents[24].

In this paper, we compare two liquid chromatography–mass spectrometry (LC–MS) techniques (direct liquid introduction, DLI LC–MS, and open-tubular liquid chromatography, OTLC–MS) for the analysis of metribuzin (MZN) and its major metabolites in plant tissues and water samples. These two LC–MS techniques are evaluated as confirmatory techniques when the presence of these metabolites is indicated by other, less-selective, detectors.

MATERIALS AND METHODS

Equipment

OTLC

The chromatographic system used in this work has previously been described[25,26]. A 10-μm I.D. fused-silica column, approximately 1.5 m in length, coated with OV-17-V was used[27,28]. The mobile phase used was methanol–water (10:90) at a flow-rate of 50 nl/min. The OTLC–MS interface used in these experiments has been described previously[26]. The interface tip temperature was 270°C.

"Minibore" HPLC

The HPLC system used for the "minibore" separations consisted of two Gilson Model 302 pumps (Gilson Medical Electronics, Middleton, WI, U.S.A.), a Gilson Model 802B manometric module, a Gilson gradient controller, and a Waters 440 UV detector (Waters Assoc., Milford, MA, U.S.A.). One Rheodyne Model 7125 injector

(Rheodyne, Cotati, CA, U.S.A.), equipped with a 1-ml loop, was placed between the column and the mass spectrometer, and was used for the introduction of a tuning solution. Two Rheodyne Model 7010 injectors were used in the sample preconcentration system, which will be described below. A DuPont Zorbax C_{18} column (Dupont Instruments, Analytical Division, Wilmington, DE, U.S.A.), 2 mm I.D., packed by Alltech Assoc. (Deerfield, IL, U.S.A.), was used for the separations. The flow-rate used was 0.2 ml/min. The solvent was methanol–water (50:50). The DLI interface was purchased from Hewlett-Packard (Palo Alto, CA, U.S.A.).

Radiochemical analysis

For the experiments involving radiochemical detection, a Flo-one radiodetector (Radiomatic Instr. and Chemical, Tampa, FL, U.S.A.), operated in the ^{14}C mode, was used. The effluent from the Waters 440 UV detector was sent into the Flo-one detector, where Hydrofluor scintillation fluid (National Diagnostics, Manville, NJ, U.S.A.) was added.

Mass spectrometry

The mass spectrometer used was a Finnigan 3300 chemical ionization mass spectrometer (Finnigan-MAT, San Jose, CA, U.S.A.), previously modified for negative ion detection[29], and DLI[30,31] and OTLC[26] probe introduction.

Samples and solvents

The metribuzin standard was obtained from the E.P.A. repository (Environmental Protection Agency, Research Triangle Park, NC, U.S.A.). Standards of the three metribuzin metabolites were obtained as a gift from Mobay (St. Louis, MO, U.S.A.).

The solvents used (HPLC-grade methanol and acetonitrile) were from Fisher Scientific (Fairlawn, NJ, U.S.A.). The water used was Millipore "Milli-Q/Milli-Rho" water (Millipore, Bedford, MA, U.S.A.).

Water samples

Water samples were collected from a lake on the N.I.E.H.S. site. Known amounts of a mixture of MZN, DA, DK and DADK were added to the lakewater to produce the spiked sample. Both the spiked and control water samples were then filtered through a Millipore HA filter (0.45 μm). For OTLC–MS analysis, 2.5-ml samples of spiked and of unspiked lakewater were freeze-dried and taken up in 10 μl methanol–water (10:90).

Plant samples

Plant culture. Three tetraploid (MZN-tolerant) soybean[5] or diploid (MZN-sensitive) soybean seeds (var. Coker 156) were planted 2 cm deep in sand in 7 cm diameter styrofoam cups. The cups were watered to field capacity and kept moist with half-strength Hoagland's solution[32] at pH 6.5. The cups were placed on a growth table for germination at ambient temperature with a fluorescent light intensity of 1500 footcandles at plant height with 14-h days. After 4 days, the seedlings had emerged. The sand was removed by washing. Seedlings selected for uniformity were placed two per flask in 250-ml Erlenmeyer flasks containing 240 ml half-strength Hoagland's

solution at pH 6.5. The flasks were wrapped in aluminum foil, and replaced on the growth table in a completely-randomized block design. At least once per day during the growth period, the volume of Hoagland's solution was measured and replaced to 200 ml.

Chemical treatment. Diploid and tetraploid seeds were treated identically. The treatments consisted of: (1) [^{14}C]carbonyl-labeled metribuzin with 4.44 mCi/mole specific activity and a purity of 98% was applied at a rate of 0.2 μCi per flask in 0.5 ml methanol to give a final concentration of 0.04 ppm in the nutrient solution; (2) unlabeled metribuzin in 0.5 ml methanol was added to the flasks to give a final solution concentration of 0.04 ppm; and (3) 0.5 ml methanol was added to the control flasks.

Chemical extraction. The two seedlings per flask were treated as a composite sample. The plants were divided into shoots and roots immediately below the cotyledonary node. Fresh weights were recorded. The shoots were homogenized in 30 ml of methanol, and the homogenates were filtered through a Buchner funnel under vacuum and washed with several volumes of methanol. The filtrates were collected and evaporated to dryness in a rotary evaporator. The extracts were resuspended in 2 ml chloroform, followed by 3 ml of methanol and refrigerated until analysis.

Sample preparation. Before LC/MS analysis, the final extracts were dried *in vacuo* and resuspended in 1 ml methanol. For DLI analysis, 100 μl of an extract was dissolved in 2.4 ml water and eluted onto the minibore column using the preconcentration/cleanup system described below. A Millipore Millex-HV (25 mm) filter was used while injecting.

For OTLC analysis, 720 μl of the 1-ml plant extract was passed through a Waters C_{18} Sep-pak, and eluted with an additional 2.5 ml methanol. The eluent was evaporated to 500 μl under nitrogen and centrifuged at 16 000 *g* for 10 min. The pellet was discarded, the supernatant was evaporated to 100 μl and centrifuged again. This was repeated for 50-μl and 20-μl volumes. The supernant was evaporated under nitrogen, resuspended in 30 μl water–methanol (90:10), and recentrifuged. The supernatant was evaporated, taken up in 10 μl water–methanol (90:10), and recentrifuged. The sample was then ready for analysis using the "dip" injection technique described below.

For radiochemical detection, the extracts from the plants which had been treated with the ^{14}C-labeled metribuzin were evaporated under nitrogen, and taken up in 1 ml methanol. A 300-μl aliquot of each sample was evaporated to 100 μl and then water (2.4 ml) was added. The extract was loaded onto the minibore column using a Millex-HV filter and the precolumn cartridge system as described below.

RESULTS AND DISCUSSION

Comparison of spectra and sensitivities (standards)

As can be seen in Figs. 2 and 3, the elution order of the compounds is different on the C_{18} minibore column and the OV-17-V OTLC column. All four of the metabolites could be separated on the DuPont Zorbax C_{18} column; three could be separated by OTLC on the OV-17-V column. The fourth compound (DADK) exhibits very low sensitivity, however, which would limit its detectability in either LC–MS mode (Table I). The detection limits of MZN, DA and DK under negative ion conditions were 10–30 pg in OTLC and 2–5 ng by DLI (Table I). Triplicate injections of MZN by

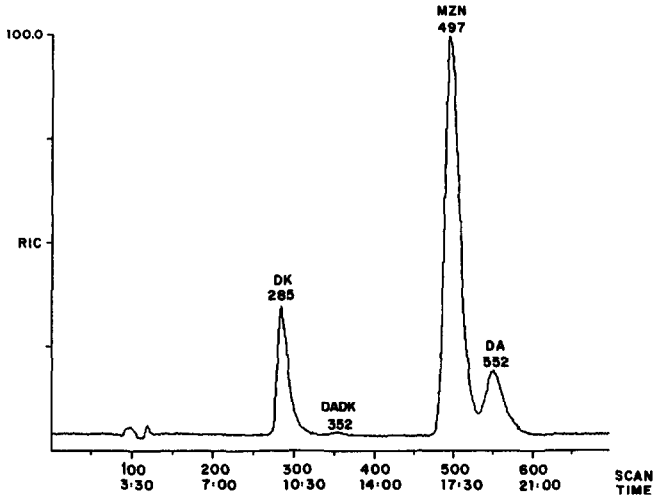

Fig. 2. Total ion chromatogram (TIC) trace for a mixture of standards by DLI LC–MS [multiple ion detection (MID), NCI]. Amounts injected were 250, 250, 250 and 500 ng of DA, DADK, DK and MZN, respectively. Time in min:s.

OTLC down to the 20 pg level are shown in Fig. 4. Since negative ion sensitivities were better than positive ion sensitivities, negative ion detection was used for the lakewater and soybean samples.

The positive and negative ion spectra obtained by OTLC–MS which uses a heated interface were essentially identical to those obtained by DLI, where the interface is cooled (Table II). No additional fragmentation was observed in OTLC–MS, thus there is no evidence of thermal degradation of these compounds.

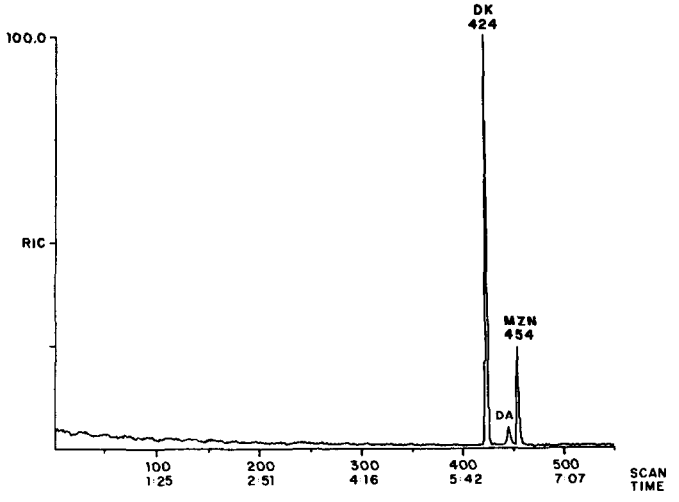

Fig. 3. Total ion chromatogram (TIC) trace for a mixture of standards by OTLC–MS (MID, NCI). Amounts injected were 200 pg each of DA, DADK, DK, and MZN.

TABLE I

DETECTION LIMITS BY MID, NEGATIVE ION MODE

Solvent, methanol–water (50:50). The compounds exhibit *ca.* 2 orders of magnitude less sensitivity in the positive ion mode, with the exception of DADK, for which the sensitivities are approximately equal.

	Selected mass (m/z)	*Detection limits (pg)*		*Relative sensitivity OTLC/DLI*
		DLI	*OTLC*	
MZN	198	2000	10	200
DA	184	5000	30	170
DK	168	5000	20	250
DADK	168	100 000	1000	100

For on-line injection of standards, the OTLC system has a sensitivity advantage of *ca.* 200:1. This can be partly explained by the absence of a solvent stream split in OTLC, and partly by the greatly reduced peak width in OTLC, leading to a higher mass flow per scan. Also, in DLI some of the sample may be lost with the solvent as the source pressure is adjusted by moving the probe inside the desolvation chamber.

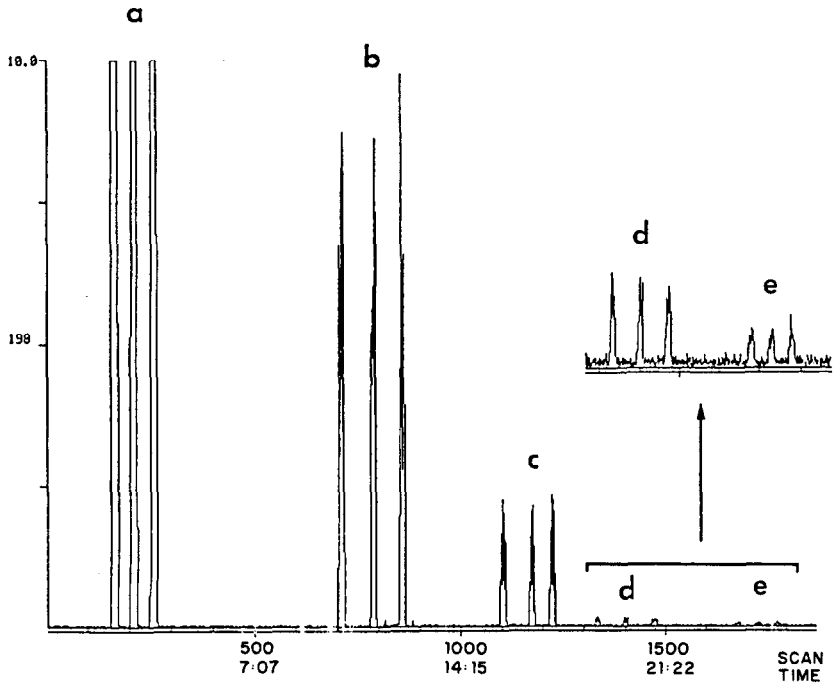

Fig. 4. OTLC–MS detection limit for MZN by MID-NCI. Triplicate injections of (a) 2 ng, (b) 400 pg, (c) 200 pg, (d) 40 pg, and (e) 20 pg MZN.

TABLE II
POSITIVE AND NEGATIVE DLI MASS SPECTRA OF METRIBUZIN AND METABOLITES
PCI = Positive ion chemical ionization; NCI = negative ion chemical ionization; RA = relative abundance.

Compound	*Mol. wt.*	*DLI-PCI-MS*			*DLI-NCI-MS*		
		m/z	*% RA*	*Proposed ID*[a]	*m/z*	*% RA*	*Proposed ID*[a]
Metribuzin	214	215	100.0	$[M+H]^+$	198	100.0	$[M-NH_2]^-$
		200	18.5	$[M-NH_2+2H]^+$	184	7.0	
		169	11.4	$[M+H-SCH_2]^+$	152	4.6	
		154	14.2	$[M-NH_2+2H-SCH_2]$			
DA	199	200	100.0	$[M+H]^+$	184	100.0	$[M-CH_3]^-$
		154	45.1	$[M+H-SCH_2]^+$			
DK	184	185	100.0	$[M+H]^+$	168	100.0	$[M-NH_2]^-$
		170	42.0	$[M-NH_2+2H]^+$			
DADK	169	170	100.0	$[M+H]^+$	168	100.0	$[M-H]^-$

[a] The proposed identifications are based only on nominal mass values.

Environmental applications

The levels of MZN and its metabolites expected to be found in treated soybean plants and in runoff studies are sufficiently low that, even with the low detection limits achieved by LC–MS, sample extracts have to be concentrated prior to analysis. This preconcentration can be done in two ways, either on-column or by solvent removal prior to injection.

Concentration/injection technique—DLI. Direct injection of the soybean plant extract onto the minibore HPLC column led to rapid degradation of column performance. Changes in the elution order of DA and MZN in soybean extracts and lakewater samples were sometimes observed. Similar effects were observed in earlier HPLC studies on these metabolites[33,34].

To avoid these matrix effects, and to improve the on-column detection limits for these compounds, a column switching technique was developed. This system is shown in Fig. 5 where two pumps delivered different mobile phases at different flow-rates to different column combinations (pump A: 100% water at 0.8 ml/min; pump B: methanol–water (50:50) at 0.2 ml/min). A Waters Guard-Pak Resolve C_{18} cartridge was used for sample preconcentration and cleanup, and a DuPont Zorbax minibore column was used for the separation. In addition to the requirements for standard HPLC work, the use of a preconcentration/cleanup system in DLI LC–MS required that constant flow be maintained to the mass spectrometer to avoid loss of the DLI jet. The procedure used is as follows (refer to Fig. 5):

(1) Loading configuration: a 2.5-ml loop was filled with the solution to be analyzed. Pump A (weak solvent) and pump B (stronger solvent) flow into the cartridge and the analytical columns, respectively. In this reversed-phase system, the weak solvent used was water (more polar), and the stronger solvent used was methanol–water (50:50) (less polar).

(2) Concentrating configuration and front-flushing: the injection valve was turned so that pump A (weak solvent) flowed through the cartridge, concentrating the

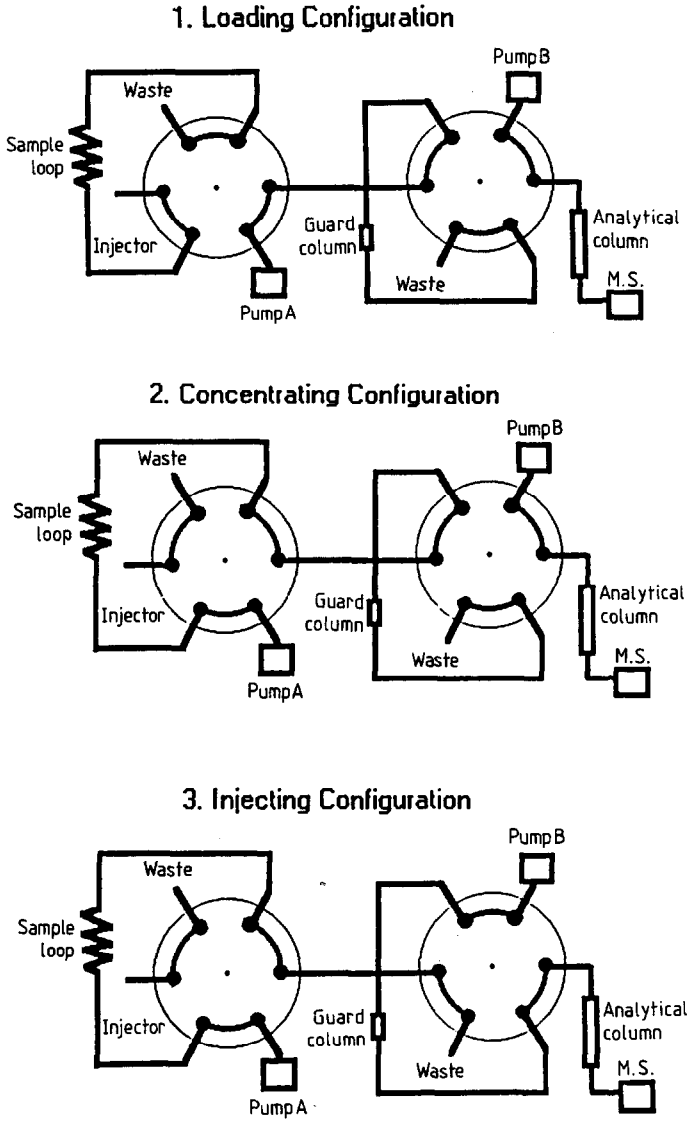

Fig. 5. On-line preconcentration/cleanup system for DLI LC–MS.

compounds of interest on the cartridge, and flushing away the more polar constituants of the matrix. The flow-rate of pump A was not restricted by the limitations of the analytical (minibore) column, so higher flow-rates could be used. Thus analysis time was reduced by reducing sample preconcentration time.

(3) Injecting configuration: after 5 min, the switching valve was turned so that the cartridge was now on-line with the analytical column. At this point the preconcentrated analytes were eluted onto the analytical column by the stronger mobile phase from pump B.

(4) Backflushing: After an additional 2 min, the two valves were turned back to the loading configuration. At this point, the position of a low-pressure three-way valve on the solvent inlet line of pump A was changed to introduce 100% methanol into the pump instead of the weak solvent, water. At this time, any less-polar compounds which may have been adsorbed onto the cartridge could be removed while the separation was accomplished on the analytical column. Approximately 10 min before the end of the run (the run duration here was approximately 30 min), the three-way valve was turned back to the weaker solvent, here 100% water.

This sequence of operations permitted the "injection" of 2.5 ml of lakewater (or 100 μl of plant extract dissolved in 2.4 ml of deionized water) onto the minibore column without loss of the analytes during the preconcentration step, without loss of resolution, and without degradation of column performance over a series of analyses.

This preconcentration/clean-up technique was equally applicable to UV or radiochromatographic detection, and should prove useful for the analysis of pesticides and other analytes for which precolumn cartridges sufficiently selective for the compounds of interest can be found. Similar column switching schemes have been used for problems as diverse as the analysis of impurities in gasoline[35] or drugs in biological fluids[36]. Recently, an off-line preconcentration/cleanup scheme was reported for a variety of pesticides prior to thermospray LC–MS analysis[37,38]. The on-line scheme described here should also be applicable to these and other pesticides and to this type of LC–MS analysis. This injection technique can be used for sample cleanup even if preconcentration is unnecesary, and could be automated if desired.

Injection technique—OTLC. The original injection system[25,26] required *ca.* 200 μl of sample solution in order to make an injection of *ca.* 1 nl. For situations where the sample volume is limited, a "dip" technique, analogous to on-column injection in capillary GC–MS, was developed in which the vacuum in the mass spectrometer was used to pull a known volume (*ca.* 1 nl) into the OTLC column. In this new technique, only 2–10 μl were needed to per injection. A brief description follows (refer to Fig. 6): the Valco four-port injection valve was turned to the "load" position to stop the mobile phase flow. The column was then disconnected from the "tee" and the end was dipped into the sample solution. At this time the analyte solution was drawn into the column by the pressure differential. After an appropriate injection time, typically 8–10 min, the column was reconnected to the "tee", the waste valve was opened, and the Valco valve is turned so that the pressurized mobile phase rinses the outer part of the column tip. After about 5–10 s, the waste valve is closed again, and the chromatographic process begins.

The amount of sample entering the column during this "dip" procedure was a function of the sample viscosity, the mobile phase viscosity, and the column taper. The amount of sample injected can be determined experimentally by measuring the response from different timed injections of a solution of known detector response and making a calibration curve. The amount can also be determined by measuring (with an optical microscope) the movement of the sample meniscus as a function of time and calculating the volume from the inner diameter of the column. Fig. 7 shows a loading curve determined for methanol–water (10:90). This figure shows no apparent movement for about 300 s, and the rate gradually increases until *ca.* 500 s. After this initial lag phase, the linear velocity throughout the column was constant.

Comparison of DLI and OTLC–MS results. A chromatogram of a diploid

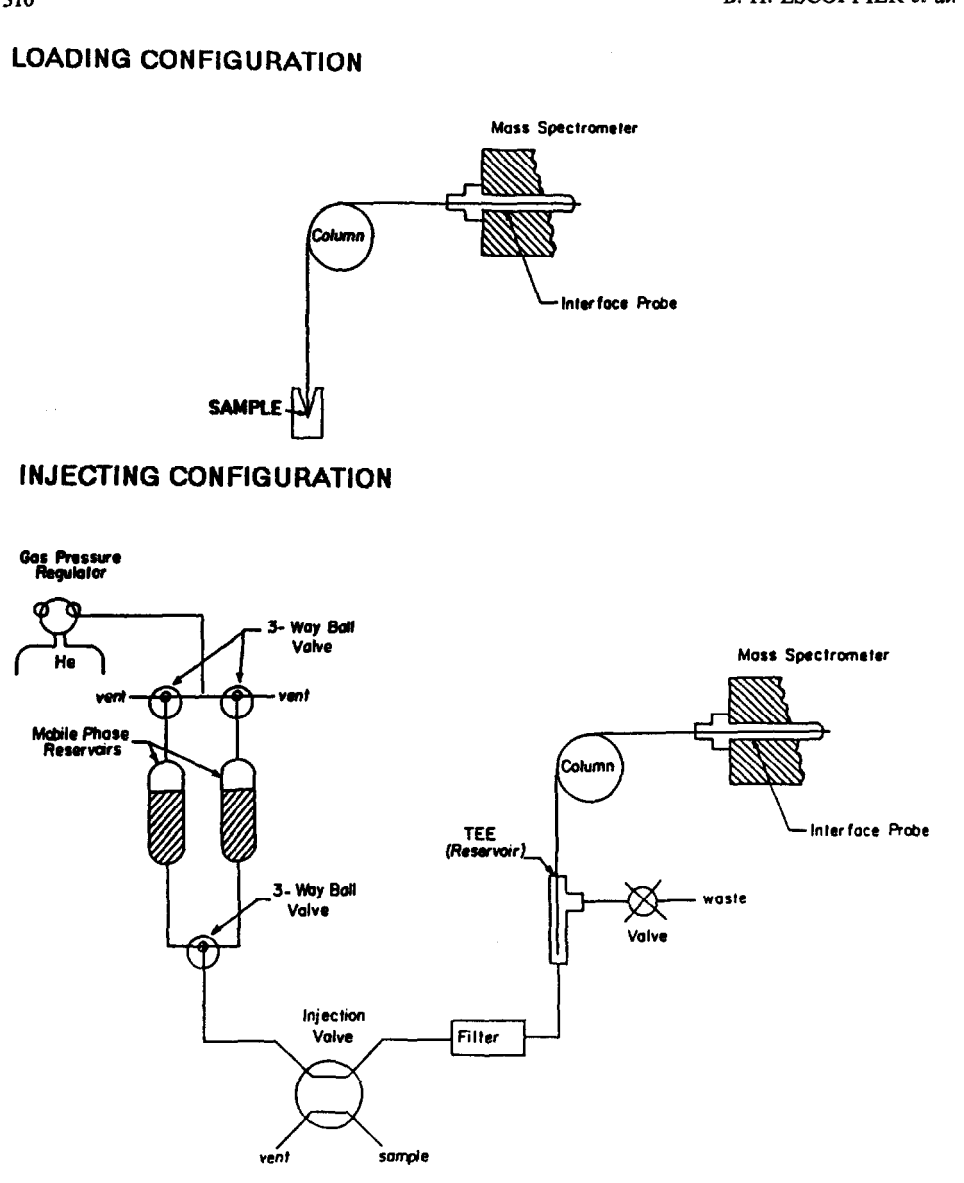

Fig. 6. Loading and injection system for OTLC–MS.

soybean shoot extract run using DLI LC–MS and the preconcentration technique is shown in Fig. 8. A DLI LC–MS chromatogram of a 2.5-ml lakewater sample spiked with 25 ng DA, DK and DADK, and 50 ng MZN [10, 10, 10 and 20 ppb (μg/l), respectively] is shown in Fig. 9.

There was very little chemical noise in the DLI chromatograms of the lakewater extracts, so the detection limits (for the amount of material reaching the source) were the same as those determined by injection of pure compounds. For the soybean extracts, the estimated source detection limits for DA and MZN were effectively the

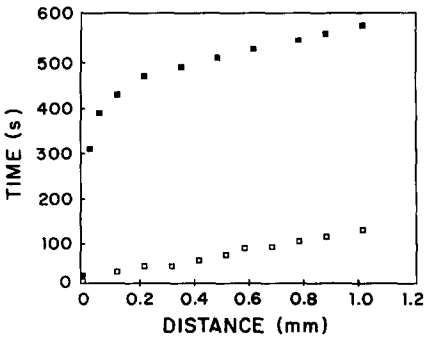

Fig. 7. OTLC solvent flow-rate, loaded by the "dip" technique. (■) = Near column entrance; (□) = in interior of column.

same as those calculated for the standard solutions. The detection limits for DK and DADK, however, were somewhat poorer in the soybean extracts due to chemical noise from other components in the matrix.

The chromatogram obtained on a diploid soybean shoot extract by OTLC–MS is shown in Fig. 10 (the DLI LC–MS results from this same extract were shown in Fig. 8). The sensitivities of both OTLC and DLI LC–MS were sufficient to allow detection in extracts of soybean plants which had been treated with 0.04 ppm MZN in the culture medium. Due to the limited injection volume of OTLC, however, the plant extract had to be concentrated by a factor of almost 100 compared to DLI before the metabolites could be detected. The plant matrix had a deleterious effect on the performance of the OTLC–MS system even with only *ca.* 0.1 nl of the concentrate injected. The matrix also

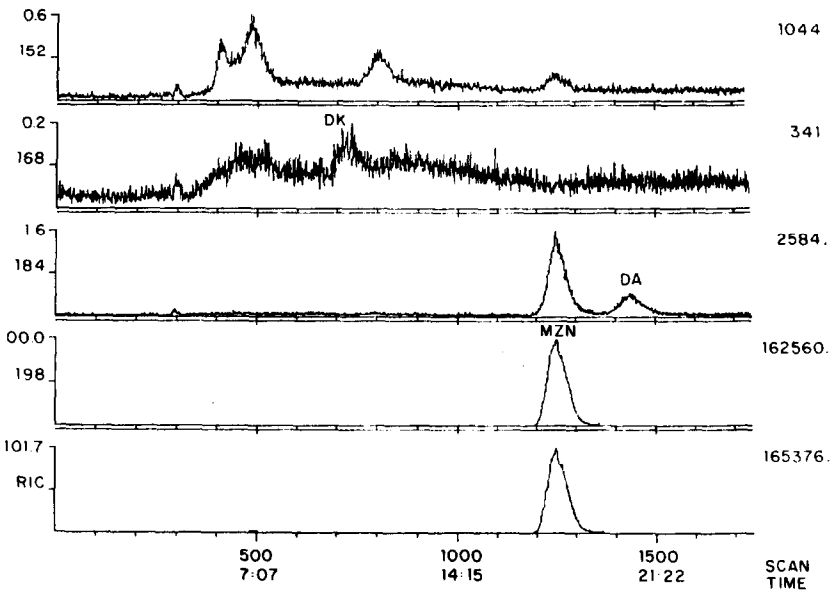

Fig. 8. Selected ion traces for a soybean shoot extract by DLI LC–MS (MID, NCI). 100 μl of 1 ml total extract injected.

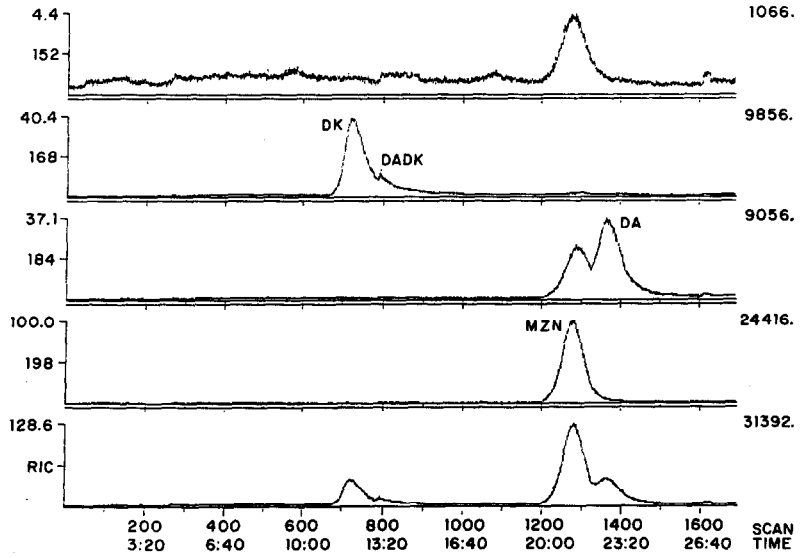

Fig. 9. Selected ion traces by DLI LC–MS (MID, NCI) for a lakewater extract spiked with DA, DADK, DK and MZN at the 10-, 10-, 10- and 20-ppb level, respectively. Results shown are for an injection of 2.5 ml of lakewater using the preconcentration/cleanup system.

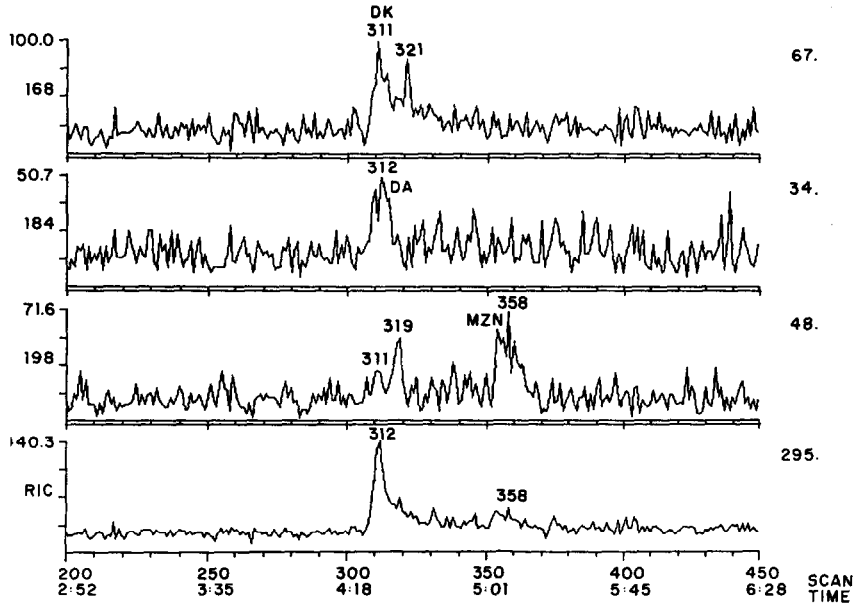

Fig. 10. Selected ion traces by OTLC–MS (MID, NCI) for the same soybean shoot extract shown in Fig. 8. Approximately 0.1 nl was injected, which corresponds to *ca.* 7.2 nl of the original 1-ml extract.

caused a shift in the retention time of DA so that it coeluted with DK in the plant sample.

Problems of column plugging occurred in the OTLC–MS analysis of the lakewater extracts (possibly due to high concentrations of inorganic salts) so no chromatograms could be obtained. Similar problems of plugging due to inorganic salts have been observed in thermospray, which also uses a heated vaporizer.

One would have expected that the sample preconcentration and the relative sensitivity advantage of OTLC over DLI would have partially compensated for the small injection volume of OTLC and would have resulted in a better response for OTLC than was observed (Table III). DLI, however, gave a much better response than did OTLC. It is possible that some of the analyte may have been lost during the extensive sample concentration process used for the OTLC samples. Matrix effects appeared to be more severe than in conventional HPLC and precluded a larger OTLC injection. Matrix effects are reduced in DLI LC–MS both by the inherent sample loading characteristics of the larger column, and by the injection system developed for on-line sample concentration and cleanup which could be used for DLI and not for OTLC.

Comparison of UV, radiochemical, and MS results. In the UV chromatogram shown in Fig. 11, the peak corresponding to the retention time of DA resulted from a component of the matrix. This and other matrix components precluded the use of UV detection for such low levels of metabolites.

The major metabolite detected by both LC–MS techniques in both diploid and tetraploid soybeans was DA. A trace of DK was also detected. The pattern of known metabolites found by LC–MS was confirmed by radiochemical detection on extracts of soybean plants treated with labeled MZN.

No consistent differences in the ratio of DA and MZN between diploid (MZN-sensitive) and tetraploid (MZN-tolerant) soybeans could be detected by DLI LC–MS, mainly due to the lack of suitable internal standards for quantitation. The major differences appared to be in the late-eluting metabolites, detected by radiochemical detection. These metabolites did not show masses characteristic of the previously-identified metabolites. The structures of these metabolites have not been determined and are the subject of continuing investigation.

TABLE III
SAMPLE LOADING COMPARISON

	DLI	*OTLC*
Volume injected	100 μl[a]	0.1 nl
Split ratio (%) (% to mass spectrometer)	<20%	100
Actual volume to mass spectrometer	20 μl[a]	0.1 nl
Relative volume to mass spectrometer	200 000:1	
Relative amount of analyte to mass spectrometer	3000:1	
Relative sensitivity	1:200	
Relative response expected	15:1	

[a] With on-line preconcentration/clean-up system.

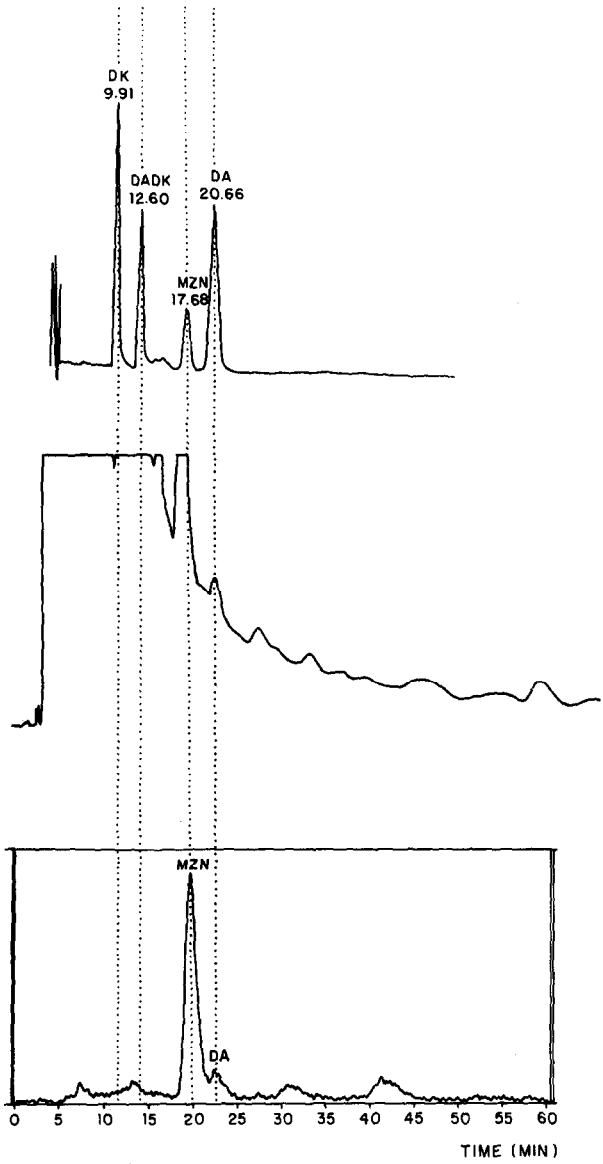

Fig. 11. A comparison of UV and radiochemical detection for the analysis of MZN and its three metabolites in soybean shoot samples. The concentrations used for the standard mixture were 250, 250, 250, and 500 ng of DA, DADK, DK, and MZN injected, respectively (detector sensitivity, 0.2 A); the UV and radiochromatograms were done on 300 μl of a 1-ml extract.

CONCLUSIONS

Several properties of OTLC–MS make it appear attractive for the analysis of environmental samples. One of these advantages is the very small amount of sample required per analysis through the use of the "dip" injection technique as demonstrated

in this paper. Compared to DLI, the OTLC interface requires no sample split, so all of the injected sample goes to the MS source. This should give better on-column detection limits. Another advantage of OTLC is better chromatographic resolution, resulting in shorter analysis times for a given separation and narrower peaks. The narrow peak width gives a higher mass flow to the MS source than do wider peaks. This should also increase the sensitivity of OTLC over DLI. Another advantage over DLI is that less mobile phase enters the MS source, so electron impact ionization is possible, and, in reversed-phase systems, less water enters the source, resulting in longer filament lifetimes.

One disadvantage of OTLC is lack of a commercial source of columns or interface probes. More important, for environmental samples (or other samples with difficult matrices), OTLC columns may be more sensitive to matrix effects, especially if extensive preconcentration of the sample is required. These matrix effects can dramatically reduce the column efficiency and chromatographic resolution. Plugging of the OTLC column and/or interface may result from matrix components or analyte solubilities; in addition, some "shedding" from the in-line filter used to protect the column may occur[39]. In the taper-type interface like that used here, when the column is plugged, the taper has to be reformed. The OTLC column can easily be overloaded and the OTLC–MS system has a low dynamic range. Successful OTLC–MS analysis requires that the amount injected onto the OTLC column be detectable by the MS. While small injection volumes (*ca.* 1 nl) mean that many analyses can be performed on a few microliter of sample, these small injection volumes also limit the amount of analyte entering the MS source. Solubility of the analyte in the mobile phase also can be a limiting factor.

Some of the problems in OTLC–MS of environmental samples could possibly have been overcome by extensive off-line sample concentration and cleanup (*e.g.*, desalting, in the case of the lakewater samples). The purpose of the present study, however, was to evaluate OTLC–MS and DLI LC–MS for the analysis of crude extracts so these additional cleanup procedures were not included. Some of the difficulties encountered in the analysis of crude extracts may be mitigated by the use of packed capillary LC columns, which are presently under development.

The preconcentration/cleanup technique, which cannot be used with the small volumes and flow-rates of OTLC, and the inherent sample loading capacities of minibore and larger columns, can combine to give DLI LC–MS an advantage over OTLC–MS for the analysis of environmental samples. MZN and its metabolites could be detected in treated soybean tissue and in lakewater at the ppb level by DLI LC–MS. Quantitation by either LC–MS technique would require coinjection of a suitable internal standard to compensate for sensitivity changes in the MS source. Without internal standards, LC–MS can be used for confirmation of the identity of suspected metabolites detected by other less-selective techniques, and the identification of new metabolites, but is less well-suited to quantification.

REFERENCES

1 R. R. Gronberg, D. R. Flint, H. R. Shaw and R. A. Robinson, *Report No. 29800*, Research and Development Department, Mobay Corp., St. Louis, MO. Agricultural Chemicals Division, Stillwell, KA, 1971.
2 D. D. Church, R. R. Gronberg and D. R. Flint, unpublished results, 1972.

3 B. E. Pape and M. J. Zabik, *J. Agric. Food Chem.*, 20 (1972) 72.
4 P. W. Albro, C. E. Parker, G. D. Marbury, O. Hernandez and F. T. Corbin, *Appl. Spectrosc.*, 38 (1984) 556.
5 E. O. Abusteit, F. T. Corbin, D. P. Schmitt, J. W. Burton, A. D. Worsham and L. Thompson, Jr., *Weed Sci.*, 33 (1985) 618.
6 D. S. Frear, H. R. Swanson and E. R. Mansager, *Pestic. Biochem. Physiol.*, 23 (1985) 56.
7 T. Zlatev and D. Stoichev, *Khranitelnoprom. Nauka*, 2 (1986) 42.
8 J. Kovac, J. Tekel and K. Kurucova, *Z. Lebensm.-Unters. Forsch.*, 184 (1987) 96.
9 H. J. Stan and D. Mrowetz, *J. Chromatogr.*, 279 (1983) 173.
10 M. Eichner and R. Renner, *Z. Lebnm.-Unters.-Forsch.*, 170 (1980) 1.
11 A. Andersson and B. Ohlin, *Vaar Foeda*, 38 (1986) 79.
12 L. Stalder and W. Pestemer, *Weed Res.*, 20 (1980) 341.
13 W. Pestemer, L. Stalder and B. Eckert, *Weed Res.*, 20 (1980) 349.
14 K. A. Krieger, D. B. Baker and J. W. Kramer, *Arch. Environ. Contam. Toxicol.*, 17 (1988) 299.
15 H. Geyer, G. Politzki and D. Freitag, *Chemosphere*, 13 (1984) 269.
16 Y. Nishiuchi, *Suisan Zoshoku*, 30 (1982) 158.
17 M. Grandet, L. Weil, K. E. Quentin, *Z. Wasser Abwasser Forsch.*, 21 (1988) 21.
18 D. Freitag, L. Ballhorn, H. Geyer and F. Korte, *Chemosphere*, 14 (1985) 1589.
19 J. W. Kramer and D.B. Baker, *ASTM Spec. Tech. Publ.*, 867 (1985) 116.
20 D. F. Brown, L. M. McDonough, D. K. McCool and R. I. Papendick, *J. Agric. Food Chem.*, 32 (1984) 195.
21 A. Kettrup, W. Maasfeld, D. Dubisch and U. Kampschultze, *Pergamon Ser. Environ. Sci.*, 7 (1982) 231.
22 T. A. Presley and J. E. Longbottom, *EPA Report, EPA-600/4-82-013, Gov. Rep. Announce Index*, 82 (1982) 1544.
23 I. A. Shevchuk, Y. G. Dubchenko, V. V. Kucherenko and Z. Gulahmad, *Ukr. Khim. Zh.* 53 (1987) 732.
24 *E.P.A. Chemical Information Factsheet for Metribuzin, Factsheet No. 53*, Office of Pesticide Programs, E.P.A. Registration Division, Washington, DC, June 30, 1985, p. 5.
25 E. J. Guthrie and J. W. Jorgenson, *Anal. Chem.*, 56 (1984) 483.
26 J. S. M. de Wit, C. E. Parker, K. B. Tomer and J. W. Jorgenson, *Anal. Chem.*, 59 (1987) 2400.
27 P. R. Dluzneski and J. W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 332.
28 P. R. Dluzneski, *Ph. D. Thesis*, University of North Carolina at Chapel Hill, 1987.
29 M. Friesen, *Ph. D. Thesis*, Kansas State University, Manhattan, KA, 1977.
30 C. E. Parker, R. D. Voyksner, Y. Tondeur, J. D. Henion, J. R. Hass and J. Yinon, *J. Forensic Sci.*, 27 (1982) 495.
31 C. E. Parker, C. A. Haney and J. R. Hass, *J. Chromatogr.*, 237 (1982) 233.
32 D. R. Hoagland and D. I. Arnon, *California Expt. Station Circular 347*, University of California at Davis, Davis, CA, 1950, 32 pp.
33 C. E. Parker, G. Degen, E. O. Abusteit and F. T. Corbin, *J. Liq. Chromatogr.*, 6 (1983) 725.
34 C. E. Parker, A. V. Geeson, D. E. Games, E. D. Ramsey, E. O. Abusteit, F. T. Corbin and K. B. Tomer, *J. Chromatogr.*, 438 (1988) 359.
35 R. C. Ludwig and R. Eksteen, *LC·GC Mag. Liq. Gas Chromatogr.*, 6 (1988) 250.
36 J. W. Veals and C. C. Lin, *Am. Lab. (Fairfield, Conn.)*, 4 (1988) 42.
37 T. A. Bellar and W. L. Budde, presented at the *35th Annual Conference on Mass Spectrometry and Allied Topics, Denver, May 24–29, 1987*, Abstract p. 1072.
38 G. A. Junk and J. J. Richard, *Anal. Chem.*, 60 (1988) 451.
39 J. A. Poppiti, personal communication.