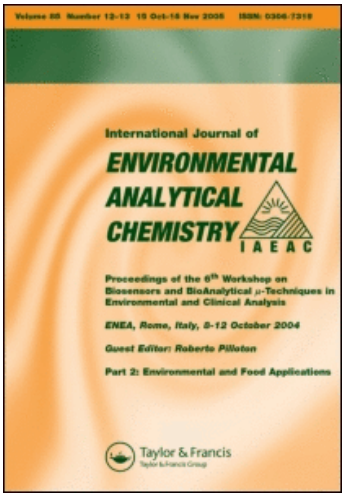

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Separation of Imazalil and T824 in Cucurbits—Part one: Comparison of HPLC Reversed-Phase Columns

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SEPARATION OF IMAZALIL AND T824 IN CUCURBITS—PART ONE: COMPARISON OF HPLC REVERSED-PHASE COLUMNS

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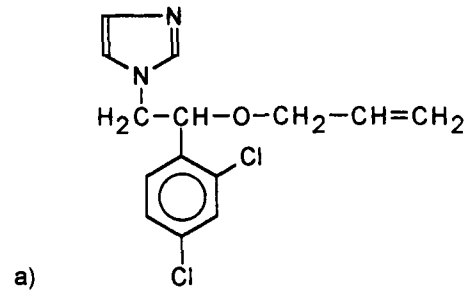
Seven reversed-phase columns, HRPB, Kromasil 100-5-C18, S5ODS2B, Vydac 218TP, YMC Polymer C18, Zorbax SBPhenyl and Zorbax SBCN, were compared with the polymeric Hamilton PRP-1 used so far, for ability to separate and determine imazalil and its degradation product, T824. Zorbax SBCN presented the best performance with sharp peak shape for both compounds, the height for imazalil being much superior to that from the PRP-1 column. It presented the best N and α under the conditions selected and gave a k′ of 4.39 and 3.00 for imazalil and T824, respectively. The retention time for T824 was the second greatest of the columns, which is likely to be advantageous for the extracts of interest. As far as time of analysis is concerned, it presented a shorter total time of ca 8 minutes using a ratio of 6:4 for the mobile phase of acetonitrile:water. That ratio was confirmed to give higher and sharper peaks for both compounds and to lead to a lower limit of detection for imazalil and for T824. The better separation obtained for imazalil and T824 with this column is attributed to the reduction of the strong adsorption of basic compounds due to the steric protection of silanol groups.

KEY WORDS: HPLC, imidazole fungicides, imazalil, T824, chromatographic separation, reversed-phase column.

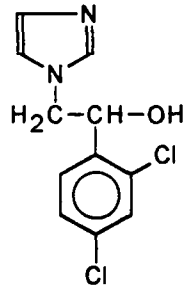
INTRODUCTION

Imazalil (Figure 1a), a fungicide used in pre- and post-harvest treatment of vegetables, has been separated on two reversed-phase HPLC columns C8[1,2] and C18[3,4,5,6] and by a normal-phase diol column[7]. However, its degradation compound T824 (Figure 1b) has only been determined using the C8 column[2].

Our work started using a polymeric column[8] which is pH stable, but unfortunately it proved to be dimensionally unstable, decreasing the column life and broadening the peak shape of the compounds of interest, thus leading to quantification errors. Decreased particle size (10 to 5 μm) was tried in order to improve separation, but this accelerated the degradation due to pressure enhancement. The limit of detection was also a problem, with broad peaks at low



a)



b)

Figure 1 Chemical formulae of (a) 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)-ethyl]-1*H*-imidazole (imazalil) and (b) 1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)-1-ethanol (*O*-dealkylated metabolite of imazalil).

concentration and high noise. So it was decided to try to find an alternative column by carrying out a comparison of imazalil and T824 separation using different columns.

MATERIALS AND METHODS

Standards: Reversed-phase Test Mix (Alltech), naphthalene, phenol, imazalil and T824. The first three solutions of standards were qualitatively diluted in acetonitrile:water (8:2). Solutions of imazalil, T824 and of imazalil together with T824 were prepared in water and mobile phase acetonitrile:water (6:4) at concentrations of 10, 5.0, 2.5, 0.5, 0.05, 0.01, 0.005 and 0.001 μg/ml.

Sample: 1 Kg of courgette obtained from a local supermarket.

Columns: HRPB, Kromasil 100-5-C18, Hamilton PRP-1, S5ODS2B, Vydac 218TP, YMC Polymer C18, Zorbax SBPhenyl and Zorbax SBCN. Table 1 shows the packing material for each column. Table 1 shows the characteristics of each column.

Table 1 Stationary Phases of the HPLC Columns Studied

Column[ab]	*Packing Material (functional group)*	*Pore Diameter (nm)*	*Particle Size (μm)*
HRPB[c]	C8-C18	11	5
Kromasil 100-5-C18[c]	C18	10	5
PRP-1	SDVB[d]	7.5	10
S5ODS2B[c]	C18	8	5
Vydac 218TP[c]	C18	30	5
YMC Polymer C18	C18-Acrylamide	–	10
Zorbax SBCN[e]	Cyanopropyl	7	5
Zorbax SBPhenyl[e]	Phenyl	7	5

[a] all columns are silica based, except PRP-1 and YMC Polymer C18
[b] column diameter/length (mm): 4.6/250
[c] fully endcapped
[d] styrene-divinylbenzene
[e] particles are produced from small extremely uniform colloidal silica microbeads, which are agglutinated in an organic polymerization process to form spherical particles

HPLC equipment: Rheodyne injector, Spectraphysics pump and Kratos Spectroflow 757 detector. The HPLC conditions used were: 202 nm, chart speed 0.5 cm/min, flow rate 1.0 ml/min, 20 μl injection volume, mobile phase acetonitrile:water (6:4) (for PRP-1 column we used 1 drop of ammonia, s.g. 0.88, per 1L). For plate number determinations, the mobile phase was acetonitrile:water (8:2).

METHODS

Using all the columns in turn, a mixture of imazalil and T824 (10 μg/mL each) was injected into the HPLC system and the peak shape, peak height, retention time (t_R), separation between the peaks (resolution, R), capacity factor (k′), and separation factor (α) were compared. Based on the conclusions of this study, the columns, HRPB, PRP-1, Vydac 218TP and Zorbax SBCN, were further compared for their plate number and imazalil and T824 separation.

PRP-1 and Zorbax SBCN

Standard and courgette extract: Using the column with best performance (Zorbax SBCN) and PRP-1, courgette extracts spiked with 2.5 and 0.5 μg/mL each of imazalil and T824 were then analysed.

Limit of detection: In order to determine the lowest concentration of the imidazoles detectable on each of the two columns, solutions at concentration of 0.05, 0.01, 0.005 and 0.001 μg/mL each were applied. Peak shape and height and noise were then compared.

Repeatability: Solutions of imazalil and T824 at concentrations of 2.5, 0.5 and 0.05 μg/mL each were injected 10 times into the HPLC system and repeatability of heights and retention times assessed.

Mobile phase study: acetonitrile:water at ratios of 7:3, 6:4, 5.5:4.5 and 5:5 were used to separate the compounds of interest (2.5 μg/mL each).

RESULTS AND DISCUSSION

Column comparison

The columns were chosen bearing in mind the need to separate basic compounds. Of the eight columns investigated, HRPB, YMC Polymer C18, Zorbax SBPhenyl and Zorbax SBCN presented better performance and peak shape than the other four columns for both compounds, Zorbax SBCN giving the highest imazalil peak, followed by HRPB. On the other hand, for T824, the HRPB peak was the highest, followed by Zorbax SBCN. Zorbax SBCN presented the highest number of theoretical plates for imazalil (7.2×10^3), followed by YMC Polymer C18 (3.8×10^3), Zorbax SBPhenyl (3.3×10^3) and HRPB (2.3×10^3). The S50DS2B column presented the worst peak shape. The PRP-1 column used hitherto presented not the lowest, but the second lowest peak for imazalil and it was the third broadest. T824 presented a very good peak shape, but the peak was not very high, being rather similar to that on Zorbax SBPhenyl, and its retention time is so close to that of the solvent as to lead to difficulties of separation when using sample extracts. The next step was to use the columns that presented better separation to check their performance and determine sensitivity and limit of detection. The columns used were HRPB, Vydac 218TP and Zorbax SBCN, always comparing them to the polymeric PRP-1. The only other polymeric column used in the preliminary study, apart from the PRP-1, was the YMC Polymer C18 and it presented very good performance. It is claimed by the manufacturer that it has C18 bonded to an acrylic polymer, allowing it to be dimensionally more stable than PRP-1, which is based on styrene and divinylbenzene and so is prone to shrink and swell in different solvents, decreasing its life. The YMC column presented a very good separation of the imidazoles, but not the best, and so, due to its high price, it was not studied further.

Number of theoretical plates (N)

As far as column performance is concerned, HRPB and Zorbax SBCN have high values for N and N/m, indicating very good performance. The opposite applies to Vydac 218TP and PRP-1. The N for the PRP-1 column roughly halved over about 700 hours of usage, whereas for the Zorbax SBCN column there was virtually no deterioration with similar usage. After such usage, the PRP-1 column can only produce reasonable peak shapes in separating the compounds of interest with the addition of ammonia. The deterioration was even faster for a PRP-1 5μm column.

Imazalil and T824 separation using four selected reversed-phase columns

As expected from the previous study, the columns tested presented different retention times and peak shapes using the same HPLC conditions. Without any doubt, Zorbax SBCN presented the best peak shape and height. The retention time for T824 was greater than with the other columns (except for Vydac 218TP), which is likely to be advantageous for the cucurbit extracts, but the total time of analysis (ca 8 min) is very acceptable. The k′ was 4.39 for imazalil and 3.00 for T824, giving a suitable ratio for separation (1 to 5)[9]. As far as separation of the two peaks is concerned, the separation factor (α) was the smallest. All the columns gave resolutions greater than 3 (Table 2), more than adequate for our purposes. From the study on imazalil and T824 separation using different ratios for the mobile phase, the ratios of 6:4 and 5.5:4.5 were the best, bearing in mind not only separation, but also limit of detection, time of analysis, and potential interference from the sample co-extractives. 6:4 presented higher peaks than 5.5:4.5. The better efficiency and selectivity obtained for imazalil and T824 with the Zorbax SBCN column is attributed to the reduction of the strong adsorption of basic side groups due to steric protection of the silanol groups[10,11,12,13]. Furthermore, it is claimed to give high quality of separation and to be robust, as it is made from small, extremely uniform colloidal silica microbeads, agglutinated in an organic polymerization process to form spherical particles with very narrow particle size and pore size distributions. The Vydac 218TP column, which is fully endcapped and has a large pore size (30 nm) (Table 1), appropriate for peptide separation, gave poor imazalil peak shape compared to Zorbax, but similar to those from PRP-1 and HRPB columns. The HRPB column, which has C18 and C8 groups and is fully endcapped, is specially designed for separation of free bases without requiring the addition of an organic modifier. Its packing is meant to decrease the interaction of basic solutes with active acidic silanol sites remaining on the silica. However, it does not have the performance of Zorbax SBCN for the imidazole compounds. It presented the highest k′ for imazalil (7.00) but with a broad peak and low k′ for T824 (2.60) with the t_R too close to the solvent front. Although it presented low efficiency, it has the highest selectivity (α) for these two compounds. A C18 column, as recommended

Table 2 Chromatographic Chracteristics of Imazalil and T824 Using Four Selected Reversed-Phase Columns

Column[a]	*Imazalil/T824*						
	t_R[b]	N[c]	N/m[d]	As[e]	k'[f]	R[g]	α[h]
1.	5.20/2.35	0.60/0.49	2.40/1.96	0.69/0.58	7.00/2.60	4.47	2.69
2.	4.08/1.55	0.46/0.59	1.82/2.37	0.84/0.84	5.43/1.38	4.96	3.91
3.	6.25/3.60	0.87/0.59	3.46/2.34	0.87/0.84	6.18/3.14	3.67	1.97
4.	4.58/3.40	2.91/1.96	11.6/7.91	1.09/0.89	4.39/3.00	3.66	1.46

[a] HRPB, PRP-1, Vydac 218TP and Zorbax SBCN column, respectively
[b] retention time
[c] number of theoretical plates $\times 10^{-3}$
[d] plates per meter $\times 10^{-3}$
[e] asymmetry
[f] capacity factor
[g] resolution
[h] separation factor

by the literature, was also used (S5ODS2), but it presented a low performance with the mobile phase chosen for the present study.

PRP-1 and Zorbax columns

Performance comparison using standards and a courgette extract: T824 chromatograms are reasonable both on Zorbax SBCN and PRP-1, but its retention time, as mentioned before, needs to be taken into account when cucurbit extracts are to be examined. From the PRP-1 column, the impurities of the cucurbit extract are eluted shortly behind the solvent front, at ca 3 to 4 min, which is the retention time of T824 on that column. Fortunately, on Zorbax SBCN, T824 runs later, at ca 5 min.

Limit of detection: As far as the limit of detection is concerned, using the PRP-1 column, the lowest concentration detected by the UV Kratos detector is 0.05 μg/mL at 0.005 AUFS. Using the Zorbax SBCN column, the same imazalil concentration can be detected at a setting of 0.02 AUFS or higher with a peak shape easy to quantify, allowing lower concentrations, say, 0.01 μg/mL to be detected at the same sensitivity. Higher sensitivity could be used as the noise is low.

Repeatability: The repeatability of separation and detection of imazalil and T824 at 0.5 (limit of determination) and 0.05 μg/mL showed the Zorbax SBCN column to be distinctly superior. T824 presented very good repeatability with CV (n = 10) of 2.5 and 1.9% for t_R and height, respectively, at 0.5 μg/mL. Imazalil for both concentrations used also presented acceptable repeatability with a CV of 12.4 and 14.9% for height (0.5 and 0.05 μg/mL), respectively and 0.2 and 0.8% for t_R (0.5 and 0.05 μg/mL, respectively). At higher concentrations, such as 2.5 μg/mL, the repeatability improved, presenting an SD of 0.02 and a CV of 0.3% for imazalil retention time.

Mobile phase study: Of the different ratios of acetonitrile:water investigated for the Zorbax SBCN column (7:3, 6:4, 5.5:4.5 and 5:5), 6:4, previously set for the PRP-1 column, gave the best separation of the two compounds, followed by 5.5:4.5, with which the time of analysis became rather extended. As far as T824 is concerned, its retention time with the ratio at 6:4 is preferred, as it then elutes at ca 5 min, after the co-extractives from cucurbits.

Figures 2a and 2b show chromatograms of imazalil and T824 using Zorbax SBCN and PRP-1 columns, respectively.

CONCLUSION

In the preliminary experiment, the best peak shape from the columns studied was produced by YMC Polymer C18 and Zorbax SBCN. Subsequently, Hichrom chromatograms for imazalil and T824 from the columns HRPB and Vydac 218TP also exhibited very good performance and so were investigated further in our laboratory, where Zorbax SBCN

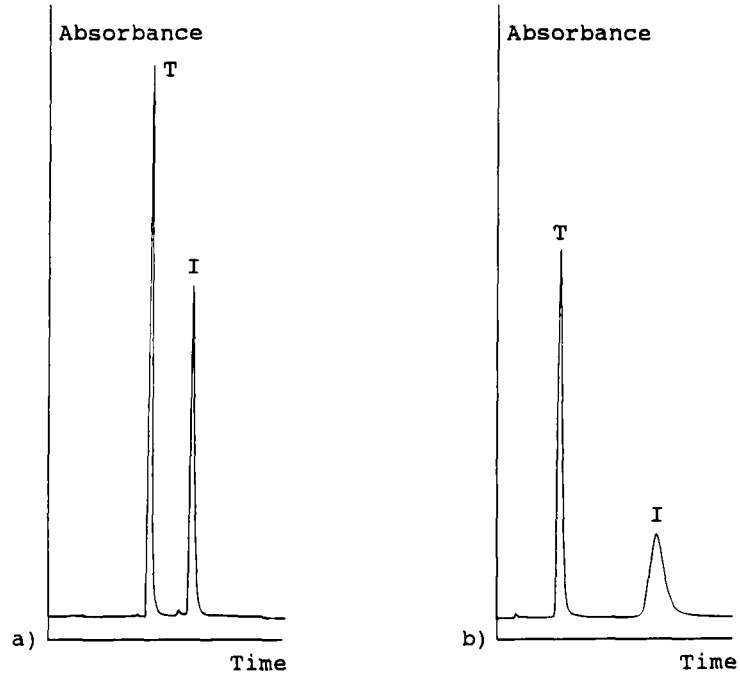


Figure 2 HPLC chromatogram of imazalil (I) and T824 (T), using reversed-phase columns: a) Zorbax SBCN and b) PRP-1. Mobile phase acetonitrile:water (6:4), 0.5 cm/min, 1.0 ml/min, 202 nm, 0.5 AUFS, 10µl loop. Note: for b) addition of 1 drop of ammonia, s.g. 0.88, to 1 L of mobile phase.

without any doubt presented the best shape and N under the conditions selected. The retention time for T824 was greater than for other columns, which is likely to be advantageous for the cucurbit extracts and the total time of analysis was an acceptable ca 8 min. As far as peak height and limit of detection are concerned, the same column produced the sharpest and therefore the highest peaks, allowing detection at lower concentrations. In addition, it is silica based, so that it is more rigid and mechanically stable than PRP-1. The retention times for both compounds, but specially for T824 (ca 5 min), are optimal, not allowing the co-extractives (at ca 3 min) to interfere. The mobile phase, acetonitrile:water, for the Zorbax SBCN column is best at a ratio of 6:4, followed by 5.5:4.5.

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References

1. J. Wynants, *Med. Fac. Landbouww. Rijksuniv. Gent*, **44,** 913–927 (1979).
2. J. Wynants, *Med. Fac. Landbouww. Rijksuniv. Gent*, **47,** 347–359 (1982).
3. S. M. Norman and D. C. Fouse, *J. Assoc. Off. Anal. Chem.*, **61,** 1469–1474 (1978).
4. G. R. Cayley; G. A. Hide and Y. Tillotson, *Pestic. Sci.* **12,** 103–109 (1981).
5. M. T. Lafuente and J. L. Tadeo, *Intern. J. Environ. Anal. Chem.*, **22,** 99–108 (1985).
6. Natural Resources Institute, Chatham Maritime, Kent, Internal information (Mr. D. Galsworthy and J. Cox) (1989).
7. P. A. Dimson and T. J. Good, *Liquid Chromatog.*, **1(1),** 28–29 (1983).
8. V. M. Scussel, Symposium on Fungicides, Herbicides and Insecticides, RSC, Analytical Division, London, UK (1992).
9. V. R. Meyer, *Practical HPLC* (Wiley and Sons, London, 1988), 310 pp.
10. J. M. Brown, Liquid Chromatography Seminar, Aldermaston, Berkshire, UK (1993).
11. J. J. DeStefano; J. A. Lewis and L. R. Snyder, *LC.GC*, **10,** 130–139 (1992).
12. J. L. Glajch and J. J. Kirkland, *LC.GC*, **8,** 1–5 (1990).
13. J. J. Kirkland; C. H. Dilks Jr. and J. E. Henderson, *LC.GC*, **11,** 291–297 (1993).