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A RAPID METHOD FOR THE QUANTITATIVE ANALYSIS OF BENOMYL ON CHERRIES BY THIN-LAYER CHROMATOGRAPHY AND *IN SITU* FLUOROMETRY*

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SUMMARY

The *in situ* analysis of benomyl (methyl 1-(butylcarbamoyl)benzimidazole-2-ylcarbamate) on thin-layer chromatograms by fluorescence scanning was investigated. A linear relationship between peak area and concentration range was found for 50–500 ng per spot, with a slight change in slope observed at higher concentrations. Concentrations as low as 20 ng per spot could be detected instrumentally, but not quantitatively. Reproducibilities of ± 10% or better can be expected in the concentration range 200–800 ng per spot. The technique was applied to the analysis of commercially treated cherries and proved sensitive to less than 1 ppm of benomyl by weight on the sample surface. This analytical approach is more rapid than other published methods.

INTRODUCTION

Benomyl (methyl 1-(butylcarbamoyl)benzimidazole-2-ylcarbamate), a systemic fungicide with broad-spectrum activity, is particularly useful in the control of storage rots of fruit and vegetables[1]. Analytical methods involving solvent extraction followed by spectrophotometric or fluorometric analysis in solution[2–4] or high-speed cation-exchange liquid chromatography[5,6] have been described. The detection and identification of benomyl and other systemic benzimidazole fungicides by thin-layer chromatography (TLC) have also been investigated[7–10], and the fluorescence spectrum of benomyl separated on silica gel thin layers has been reported[11]. However, no quantitative investigations were undertaken.

Benomyl is presently recommended in the tree-fruit-growing districts of British Columbia as a pre-harvest spray or as a post-harvest dip treatment for the control of brown rot[12]. A suitable method for the rapid quantitative determination of benomyl residues on treated cherries was therefore required. Published methods were found to be too time-consuming[2–4] or to require equipment not at present available in our

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laboratory[5,6]. TLC and *in situ* fluorometry appeared to offer the advantages of speed, simplicity and sensitivity needed, so this analytical technique was chosen for investigation.

EXPERIMENTAL

Analytical standards of benomyl and 2-aminobenzimidazole (2-AB), a degradation product to which benomyl is often converted for analysis [2,3], were obtained from DuPont (Wilmington, Del., U.S.A.). Solvents used included dichloromethane (Nanograde®; Mallinckrodt, St. Louis, Mo., U.S.A.), toluene (pesticide quality; MC/B Manufacturing Chemists, Norwood, Ohio, U.S.A.), diethyl ether (ACS grade; J. T. Baker, Phillipsburg, N. J., U.S.A.) and ethyl acetate (MC/B; pesticide quality).

Sweet cherries that had received post-harvest spray treatment with Benlate 50% WP (wettable powder) were provided by Kelowna Growers' Exchange (Kelowna, B.C., Canada). The treatment rate was 4.8 oz. of Benlate 50% WP in 50 gallons of water per 10,000 lb. of cherries (*i.e.*, approximately 0.6 g per litre per 20 kg of cherries); an untreated check sample of cherries was also provided with each set of treated cherries. The cherries were stored in a freezer until analyses were performed.

Cherries (500 g) were washed with two 50-ml portions of ethyl acetate or dichloromethane to remove surface deposits of benomyl. For initial studies with the check samples, the volume of extract was reduced to 25 ml using a Büchi Rotavapor-R rotary evaporator equipped with a Buchler water booster. 1.0-ml aliquots were then spiked with the pure analytical standard of benomyl at levels of 2–80 ng/μl. Aliquots were also spiked with 2-AB and a mixture of 2-AB and benomyl.

TLC was carried out on pre-coated (250-μm) silica gel G plates (Uniplate®; Analtech, Newark, Del., U.S.A.), which were pre-washed in the solvent system and then activated at 100° for 1 h. A 10-μl aliquot of each extract was spotted with a micropipette (Microcap®; Drummond) in all experiments, with a total of 9 spots per plate. Chromatograms were developed for 10 cm in diethyl ether–toluene (1:3) and then dried in a stream of cool air until all visible traces of solvents were removed. The developed plates were examined in a chromatogram-viewing cabinet equipped with a UVSL-58 multi-band UV lamp (Ultra-Violet Products, San Gabriel, Calif., U.S.A.).

The chromatograms were scanned for fluorescence using a Turner 111 filter-fluorometer fitted with a Camag TLC scanner at the scanning-speed setting of 1. The light source was a germicidal lamp (primary line, 254 nm). A 7-54 filter was used in the excitation beam, and a 2A filter was used in the emission side. The scans were recorded on the × 1 setting of the fluorometer using a Leeds & Northrup Speedomax H 1-mV recorder at a chart speed of 30 in./h. A precision resistor was used to lower the normal 10-mV output of the fluorometer to 1 mV. Peak areas were evaluated with a planimeter (Keuffel and Esser Co.).

The linearity of response for cherry extracts containing 2–80 ng of benomyl per μl was investigated. Studies were carried out to determine the change in fluorescence intensity of the chromatographed benomyl with time. The reproducibility was determined for each concentration level by analyzing plates containing 9 replicates of the spiked extracts. A minimum of 4 plates was measured at each concentration and the results were averaged. The effect of heating the plate before fluorescence measurement as suggested by Mallet *et al.*[11] was also investigated.

The same methodology of a surface wash with dichloromethane followed by volume reduction to 25 ml was also used for the commercially treated sweet cherries. Two replicate 10-μl aliquots from each sample were then spotted on a silica gel plate as described, together with standards prepared by spiking extracts from the controls with benomyl. Following chromatography and fluorescence scanning of the developed plate, a calibration curve was plotted for the standards. Each pair of replicates from the unknowns was averaged, and the average peak areas were then used to determine concentrations in the unknowns from the calibration curve of peak area *versus* concentration.

RESULTS AND DISCUSSION

Several solvent systems suggested in other publications[6–9] were tested for the TLC separation of benomyl from its artifacts, but the diethyl ether–toluene (1:3) system was the most satisfactory; benomyl had an R_F of 0.50, while 2-AB remained at the origin. Standard solutions and extracts were originally prepared in ethyl acetate, but benomyl was unstable in this solvent, even under refrigeration and in darkness. Dichloromethane was then tested and much better stability was found (2-AB, however, is practically insoluble in dichloromethane).

The stability-with-time study revealed little change in the fluorescence intensity of a 0.6-μg spot of chromatographed benomyl over a 6-h test period. There was an initial increase in fluorescence between the first and second readings, but this was attributed to drying of the plate. Any toluene remaining in the thin layer was found partially to quench the fluorescence; however, drying in a stream of cool air for 1 h before measurement effectively removed this interference.

The linearity of response was tested for concentrations between 20 and 800 ng per spot. Concentrations higher than 800 ng gave a signal that went off the scale of the recorder. To avoid reducing the signal from low concentrations, as would happen if the span were adjusted to accomodate higher concentrations, 800 ng per spot was chosen as the maximal concentration for this study. A typical calibration graph for cherry extracts spiked with benomyl is shown in Fig. 1.

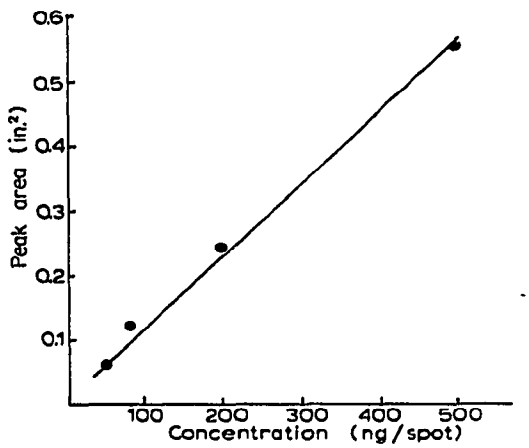

Fig. 1. Calibration graph for benomyl analysis showing linear relationship between peak area and concentration.

A linear relationship was shown to exist from 50–500 ng per spot. Above 500 ng per spot, a slight change in slope was observed, which should not in practice prevent the use of the method at concentrations up to 800 ng per spot. Ideally, standards should be chosen to bracket the concentration range of the unknowns, and a calibration graph should be plotted for each plate.

The practical lower working limit is about 50 ng per spot in an extract; 20 ng per spot were detectable, but not quantitatively (2 ng per μl spotted in a 10-μl aliquot). Although heating the plate as suggested by Mallet *et al.*[11] appeared to improve the sensitivity, it was impracticable with the cherry extracts. The heating procedure (200° for 1 h) resulted in the formation of a number of fluorescent spots from the co-extractives. Several of these were located sufficiently near the benomyl spot to produce interference. Without heating, none of these spots was visible or instrumentally detectable.

The reproducibility studies (see Table I) indicated that analyses may be carried out with a relative standard deviation of about 10% between 200 and 800 ng of benomyl per spot; a minor modification to the instrument, to be reported separately[13], gave an improvement in these results.

Experiments with commercially treated sweet cherries indicated that the method may be used to detect benomyl residues quantitatively. Two sets of cherries were analyzed, with two replicates of each sample (plus a series of spiked extracts for a calibration graph) on each plate; the results of the analyses are shown in Table II.

TABLE I

REPRODUCIBILITY OF ANALYSIS OF BENOMYL IN CHERRY EXTRACTS (9 SPOTS PER PLATE)

Concn. (ng)	*No. of plates*	*Range of relative standard deviations (per plate) (%)*	*Average relative standard deviations (per plate) (%)*
800	4	6.5–10.6	7.9
500	6	7.2–15.5	11.3
200	8	7.1–24.0	13.6 (8.9)*

* Average of 8.9% relative standard deviation was obtained for 3 plates measured following a modification to the scanner.

Standard deviation, $\sigma = \sqrt{\frac{\sum_{i}^{n} (x_i - \bar{x})^2}{(n-1)}}$

Relative standard deviation (%) = $(\sigma/\bar{x} \times 100)$, where $\bar{x}$ is the mean value for the set.

TABLE II

ANALYSIS OF BENOMYL ON COMMERCIALLY TREATED SWEET CHERRY SAMPLES

Sample	*Peak area* (in.²)*	*Benomyl per 10-µl extract (ng)*	*Concn. of benomyl on cherries (ppm)*
Treated cherries No. 1	0.06	50	0.25
Treated cherries No. 2	0.60	470	2.35

* Average of two replicates.

Based on the treatment rate, levels of up to 15 ppm of benomyl by weight could occur on the cherries at the time of application. Canadian regulations have established a tolerance of 5.0 ppm of benomyl on sweet cherries at the time of sale. Thus, a rapid method applicable in the concentration range 1–15 ppm of benomyl is desirable. The proposed method meets this requirement, with a total time of about 3 h elapsing from the beginning to the completion of an analysis, as opposed to several days for other methods[2,3,5]. Several analyses can, of course, be carried out simultaneously.

CONCLUSIONS

The combination of TLC and *in situ* fluorometry provides a simple and rapid method for the analysis of benomyl residues on cherries. The method could easily be extended to analyze methyl benzimidazol-2-ylcarbamate, a fungicidally active degradation product of benomyl, by modifying the chromatographic system and including methyl benzimidazol-2-ylcarbamate standards with the benomyl standards. The similar fluorescence properties of the two compounds would enable them to be determined simultaneously. The method offers a considerable saving in time over the previously described spectrophotometric methods[2–4] and the high-speed liquid chromatography method[5].

REFERENCES

1 H. Martin (Editor), *Pesticide Manual*, British Crop Protection Council, 3rd ed., 1972, p. 31.
2 H. L. Pease and J. A. Gardiner, *J. Agr. Food Chem.*, 17 (1969) 267.
3 H. L. Pease and R. F. Holt, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1399.
4 N. Aharonson and A. Ben-Aziz, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1330.
5 J. J. Kirkland, R. F. Holt and H. L. Pease, *J. Agr. Food Chem.*, 21 (1973) 368.
6 J. J. Kirkland, *J. Agr. Food Chem.*, 21 (1973) 171.
7 F. G. von Stryk, *J. Chromatogr.*, 72 (1972) 410.
8 P. B. Baker, J. E. Farrow and R. A. Hoodless, *J. Chromatogr.*, 81 (1973) 174.
9 P. B. Baker and R. A. Hoodless, *J. Chromatogr.*, 87 (1973) 585.
10 G. H. Tjan and L. J. Burgers, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 223.
11 V. Mallet, D. Surette and G. L. Brun, *J. Chromatogr.*, 79 (1973) 217.
12 *Tree-fruit Production Guide for Interior Districts*, British Columbia Department of Agriculture, Victoria, B.C., 1974.
13 M. Hikichi and J. D. MacNeil, *J. Chromatogr.*, submitted for publication.