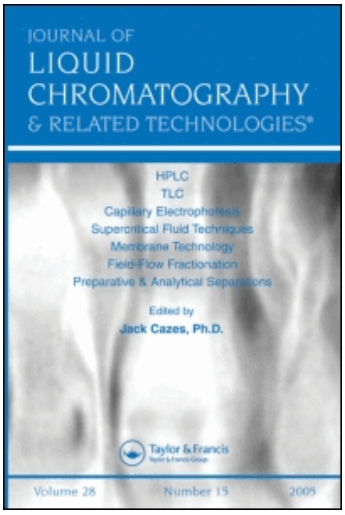

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Determination of Biphenyl in Citrus Fruits by Quantitative Thin-Layer Chromatography

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DETERMINATION OF BIPHENYL IN CITRUS FRUITS BY QUANTITATIVE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Residues of the fungicide biphenyl in citrus fruits have been determined by direct scanning of spots on phosphor-impregnated high performance silica gel TLC plates under UV light. Biphenyl was separated from fruit tissue by steam liquid-liquid extraction. Recoveries from spiked samples ranged from 92-99% at 100, 50, and 10 ppm levels. The precision of the TLC determination and overall procedure are shown to be adequate for residue analysis.

INTRODUCTION

The official AOAC method for determination of residues of the fungicide biphenyl in citrus fruits is based on steam distillation, preparative thin layer chromatography (TLC), scraping and elution of biphenyl zones from the thin layer plate, and solution UV spectrometry at 218 nm (1,2). This paper reports a more rapid, simplified method for this analysis based on the direct scanning of the UV absorbance of biphenyl spots on phosphor-impregnated high performance silica gel layers. Precision (reproducibility) and accuracy (recovery) are at least comparable to the more laborious official method, and the specificity offered by the thin layer separation is retained.

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EXPERIMENTAL

The preparation of citrus samples and biphenyl extraction using the SGA Scientific, Inc., No. JM-8590 lighter-than-water volatile oil trap were carried out as described in the official method (1,2) with the following exceptions. Extraction was performed for 3 hours at the two highest spike levels and for 4.5 hours at the lowest level. The solution was boiled vigorously during extraction and a rapid cooling-water flow was used in the condenser to prevent loss of biphenyl out of the top of the apparatus. The final heptane solution was freed from water by passing through Whatman phase separating paper (1PS) rather than a column of anhydrous Na_2SO_4.

Samples were fortified by adding 1.00 ml of ethanolic spiking solution to 100 g of blended, peeled orange or lemon fruit or ground peel in the one liter round bottom flask. The spiking solution contained 1.00 g biphenyl per 100 ml for preparation of the 100 ppm sample, 0.500 g per 100 ml for the 50.0 ppm sample, and 0.100 g per 100 ml for the 10.0 ppm sample.

TLC was carried out on 20 x 10 cm Whatman HP-KDF high performance silica gel plates. These plates contained a fluorescent phosphor that was activated by 254 nm UV light, and were divided into nineteen lanes of 8 mm width. Plates were cleaned by predevelopment with methanol-chloroform (1:1 v/v) and dried in a fume hood before use.

Biphenyl standard solutions were prepared in n-heptane at concentrations of 0.125, 0.250, 0.500, 1.00, 1.50, and 2.00 µg/µl. Standards and samples were applied to separate lanes, 2 cm up from the bottom of the plate, using disposable 4.00 µl Drummond microcap micropipets. After air drying, the layer was developed with n-heptane in a filter paper lined glass, rectangular HPTLC tank (Fotodyne) that had been pre-equilibrated with solvent for at least 10 minutes before inserting the plate.

The chromatogram was air dried in a hood and biphenyl spots were measured by scanning with a Kontes Model 800 fiber optics

densitometer equipped with a Hewlett Packard Model 3390A calculating integrator/recorder. Scanning was done in the single beam, transmission mode using the 254 nm shortwave-UV cobalt glass filter.

Precentage recovery was calculated by comparing the area of the sample zone to the area of the standard zone on the same plate representing the theoretical amount for 100% recovery. The final sample solutions were collected in 10.0 ml volumetric flasks. Sample volumes spotted and the theoretical weights representing 100% recovery were as follows for the three fortification levels: 100 ppm sample, 4.00 µl, 4.00 µg (4.00 µl of the 1.00 µg/µl standard); 50.0 ppm sample, 4.00 µl, 2.00 µg (4.00 µl of the 0.500 µg/µl standard); 10.0 ppm sample, 20.0 µl, 2.00 µg. Samples and standards were applied on adjacent lanes in duplicate, and the average area of the standard spots was compared to the two individual sample spot areas.

RESULTS AND DISCUSSION

Development with heptane porvided tight, circular zones of biphenyl with an R_F value of ca. 0.5, which is within the optimum range for quantification by densitometry (3). Approximately 12 minutes was required for a 6 cm development distance. The spots were detected as dark, absorbing zones against a bright, fluorescent background (fluorescence quenching) when the plate was viewed under shortwave UV light. The zones were measured by scanning using the 254 nm filter over the densitometer light source.

To determine that analyses were being conducted within a linear calibration region, 0.5-8 µg amounts of standard biphenyl were spotted in 4 µl volumes, and scanned. Plots of peak area vs. weight had an average correlation coefficient (R) of 0.982 and a range of 0.991-0.967 (9 replicates). Recovery values were calculated by comparison of samples to single standard zones (4 µg and 2 µg), which were within the linear calibration range.

Reproducibility of the TLC determination was measured by spotting seven 2 µg (4 µl) spots in adjacent lanes and scanning

TABLE 1

Biphenyl Recovery from Orange Fruit

Trial	Average Recovery
100 ppm Spike	
1	97.9
2	95.1
3	94.9
50.0 ppm Spike	
1	93.5
2	96.5
3	92.4
4*	95.0
10.0 ppm Spike	
1	97.9
2**	97.9
3	97.7

* orange peel

** lemon fruit

the developed chromatogram. The relative standard deviation (coefficient of variation) of the peak areas was 2.76%, which is excellent precision considering the possible combined inconsistencies resulting from plate production, sample application, mobile-phase development, and scanning.

The recovery values obtained at the same three concentrations as used in the collaborative study (1) of the official method are summarized in Table 1. Only a limited number of

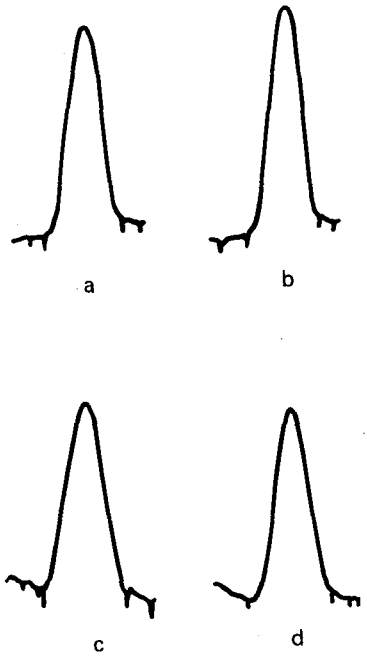

Figure 1. Densitometer scans of spots from duplicate 2.00 µg (4.00 µl) standards (a and b) and duplicate 20.0 µl extracts of the 10.0 ppm fortified lemon sample (c and d). Peak c represents 98.8% recovery and peak d 97.0%, compared to the average area of the standards.

trials were performed on three sample types because the purpose of this research was not to confirm the applicability of the sample-preparation method but to demonstrate the efficacy of the TLC determination. All of the results in the table are for fortified peeled orange fruit, except the one value for orange peel and one for lemon fruit. The average recovery results range from 92.4 to 97.0%. The somewhat higher levels of recovery at the lowest level suggest that the longer extraction time may also be beneficial at the higher levels. The percentage difference between the two sample spots for the nine experiments averaged 2.81%, which is another indication of the satisfactory precision of the TLC determination. The agreement among the

trials within each concentration level illustrate the precision of the overall procedure. The results compare favorably both in accuracy and precision with those obtained in the collaborative study (1) of the official method. Recoveries above 100% at 100 ppm were not obtained, nor were recoveries proportional to the spiking level, as in this study (1).

A blank extraction of both fruit types was carried out, and chromatograms of these extracts contained no detectable spot at the R_F value of biphenyl. Therefore, correction of the data was not necessary. Figure 1 shows typical densitometer scans of duplicate sample and standard spots used to calculate the results in Table 1.

High performance layers were chosen after determining that biphenyl zones were less diffuse and darker than on either conventional silica gel or on C_{18} chemically bonded reversed phase layers. The latter were developed with methanol-water (85:15 v/v) to provide an R_F value of 0.26 for biphenyl. Preadsorbent layers could not be used because biphenyl spotted on the preadsorbent was not consistently detected after development, indicating either loss by volatilization or irreversible sorption in the spotting area.

CONCLUSION

The above results illustrate that the official AOAC analytical method for biphenyl residues in fruit can be improved by replacing the scraping and elution of TLC zones and solution UV spectrometry by *in situ* measurement of zones directly on the thin layer plate. The changes, along with the ability to analyze multiple samples at the same time under identical conditions and to process standards in parallel, result in greater convenience and saving of time without loss of accuracy or precision. The revised method is applicable to any samples that can be successfully analyzed by the AOAC method.

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