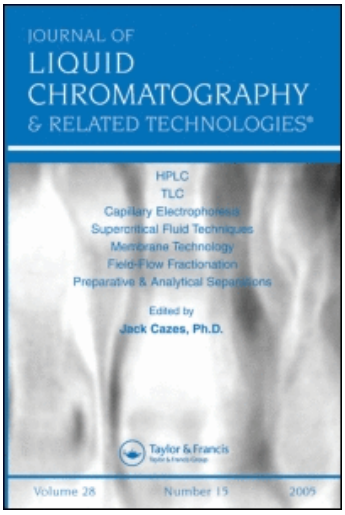

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QUANTITATIVE HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF ORGANIC ACID PRESERVATIVES IN BEVERAGES

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ABSTRACT

A simple quantitative HPTLC method for the determination of sorbic acid, benzoic acid, and dehydroacetic acid in beverages without extraction or cleanup is described. Aliquots of samples and standards are chromatographed on preadsorbent silica gel or C-18 bonded silica gel plates containing fluorescent indicator, and the zones, which quench fluorescence, are compared by scanning densitometry. Recoveries of the acids from wine and juices spiked at 50-300 ppm averaged 98%, and the coefficient of variation of replicate analyses ranged from 2-5%. Commercial sodas containing unknown amounts sodium benzoate and iced teas containing potassium benzoate were analyzed by the method, and the tea analyses were validated by standard addition. The advantages of the TLC method relative to current HPLC and absorption spectrometric procedures are described.

INTRODUCTION

Sorbic acid and benzoic acid are antimicrobial agents that are used in a great variety of foods including juices, iced teas, sodas, and other beverages (1), and sorbic acid (2) and dehydroacetic acid (DHA) (3) have been used as preservatives in wine. The latest methods for quantitative determination of sorbic and benzoic acids in foods and DHA in wine have been based on high performance liquid chromatography (1,3). The AOAC official methods for sorbic acid in wine involve steam distillation followed by UV absorption spectrometry at 260 nm or reaction with thiobarbituric acid and colorimetry at 532 nm (2,4). The only quantitative TLC method for determination of these preservatives was reported for sorbic and benzoic acids in mayonnaise and ketchup by steam distillation, solvent extraction, separation on a CN-bonded silica gel layer with fluorescent indicator, and detection under 254 nm UV light (5). In this paper we report a simple quantitative procedure for sorbic and benzoic acids in fruit juice, iced tea, and soda and sorbic acid and DHA in wines that involves direct spotting of samples on high performance preadsorbent silica gel or C-18 bonded silica gel layers containing fluorescent indicator, and scanning densitometry of quenched zones. The method was validated using juice, iced tea, and wine fortified with 50-300 ppm of the acids, and it was used to analyze commercial iced teas and sodas containing potassium sorbate and sodium benzoate, respectively, added as a preservative.

EXPERIMENTAL

Standards

Standards of sorbic, benzoic, and dehydroacetic acids were obtained from Aldrich (Milwaukee, WI). Stock

standard solutions were prepared containing 25.0 mg/ml of sorbic acid in ethanol, 20.0 mg/ml of benzoic acid in ethanol, and 20.0 mg/ml DHA in acetone. TLC standards were prepared by exact 1:100 dilution of the sorbic acid stock solution with ethanol (250 ng/ul), and 1:10 dilution of benzoic acid and DHA with ethanol and acetone, respectively (2.00 ug/ul).

Thin Layer Chromatography

Normal phase TLC was carried out on 20 x 10 cm Whatman (Clifton, NJ) LHPKDF preadsorbent high performance silica gel plates containing 19 lanes of 9 mm width. Reversed phase TLC was performed on 20 x 20 cm Whatman LKC18F preadsorbent C-18 bonded silica gel layers. Both layers contained a phosphor that fluoresced when irradiated with 254 nm UV radiation.

Sample and standard zones were applied to the preadsorbent as vertical streaks using a 10 ul Drummond (Broomall, PA) digital microdispenser. After spotting, initial zones were thoroughly dried with warm air from a hair dryer; application of sample aliquots greater than 10 ul was facilitated by positioning the hair dryer so that a gentle stream of warm was sweeping over the preadsorbent during the spotting process. Plates were developed for a distance of 7 and 10 cm beyond the sorbent-preadsorbent interface for silica-gel and C-18, respectively, in one compartment of a Camag (Wilmington, NC) twin-trough chamber with a sheet of Whatman No. 1 chromatography paper dipping in a pool of mobile phase in the other compartment in order to saturate the chamber with solvent vapors. The mobile phases were n-pentyl formate-chloroform-formic acid (2:7:1) for silica gel (6) and methanol-0.5 M NaCl (1:1) for C-18 bonded silica gel. Chromatograms were air dried in a fume hood for 20 min,

and acids were detected under 254 nm UV light in a viewing cabinet as dark quenched zones on a bright fluorescent background. Areas of zones were evaluated by scanning each lane using a Shimadzu (Columbia, MD) model 930 densitometer with a UV (deuterium lamp) source and a slit size of 6 mm (height) x 0.4 mm (width), operated in the single beam, reflectance mode. Scanning was performed at the wavelength of maximum absorption, which was determined by measuring the in situ spectrum of a standard zone of each analyte between 200 and 370 nm. Calibration curves were obtained by chromatographing 0.50, 1.00, 2.00, 4.00, 6.00, and 8.00 ul aliquots of TLC standards (125-2000 ng for sorbic acid and 1.00-16.0 ug for benzoic acid and DHA) and calculating the linear regression equation from the scan area and weight data by use of a computer program.

Analysis of Samples

Fortified samples of sorbic acid were prepared at nominal concentrations of 300, 250, and 50.0 ppm by combining 1.20, 1.00, and 0.20 ml of stock standard solution with 98.8, 99.0 and 99.8 ml of beverage, respectively. Fortified samples of benzoic acid and DHA were prepared in volumetric flasks at 250 and 125 ppm by combining 1.25 ml of stock solution with 98.75 and 198.75 ml of beverage, respectively.

Recovery analyses were performed by spotting duplicate aliquots of fortified beverage, duplicate aliquots of standard solution that contained the theoretical weight for 100% recovery and was within the linear calibration range, and duplicate aliquots of blank (unfortified) sample. For analysis of beverages spiked with 250 ppm of sorbic acid, 2.00 ul aliquots of standard, sample, and blank were compared; the standard

and sample would each contain 500 ng of acid if recovery was 100%. For wine containing 300 ppm of sorbic acid, 1.67 ul of spiked sample and blank were spotted along with 2.00 ul of standard. For analysis of a beverage spiked with 50.0 ppm of sorbic acid, 2.00 ul of standard was compared to 10.0 ul of sample. For analysis of benzoic acid or DHA spiked at 250 and 125 ppm, 1.00 ul of standard (2.00 ug) was compared respectively to 8.00 and 16.0 ul of sample. Percent recovery was calculated by comparing the average scan area of acid zones in the beverage chromatograms with the average of the standard scans.

Commercial samples of iced tea containing unspecified amounts of potassium sorbate and sodas containing sodium benzoate as preservatives were analyzed by spotting 0.50 to 8.00 ul aliquots of standard as described above adjacent to 0.50, 1.00, 2.00, 4.00, 8.00, 12.0, and 16.0 ul aliquots of sample. The soda samples were initially decarbonated by stirring and then adjusted to a pH of 2.0-2.5 by addition of concentrated HCl to assure the conversion of benzoate to benzoic acid. The ratio of the average areas of the most closely matching sample and standard zones (spotted in duplicate) was used to calculate the weight (ug) of acid in the sample; as an example, in the analysis of diet cola, the 0.5 ul benzoic acid standard zone was compared to the 8.00 ul sample zone. The weight of acid was divided by the aliquot volume of the sample (ml) to calculate the concentration as ppm. The results for the tea analyses were validated by fortifying the unknown samples with an additional 250 or 125 ppm of sorbic acid and chromatographing the fortified and nonfortified samples in parallel. The percent recovery of the added sorbic acid was calculated as 100 times the difference between the area of the fortified sample zone and the area of the unfortified

sample zone, divided by the area of a standard zone whose weight represented the amount of the standard addition.

RESULTS AND DISCUSSION

Sorbic and benzoic acids and DHA were detected as quenched zones on plates containing a fluorescent phosphor.

The limit of detection was ca. 100 ng for sorbic acid and 1000 ng for benzoic acid and DHA. The lower absolute sensitivity for densitometry of sorbic acid led to a correspondingly lower analysis limit on a concentration basis for the acid in beverages.

On preadsorbent silica gel layers developed with the formic acid solvent, sorbic, benzoic, and dehydroacetic acids formed flat, compact zones with respective R_f values of 0.61, 0.58, and 0.78. On C-18 layers developed with methanol-aqueous NaCl, sorbic and benzoic acids had respective R_f values of 0.44 and 0.59. The ability to separate sorbic and benzoic acids makes the C-18 layer the choice for analysis of products containing both of these preservatives.

The wavelengths used for densitometric quantification, as determined from the maxima of in situ spectra recorded for standard zones, were 230 nm for benzoic acid and 285 nm for sorbic acid and DHA. In addition to the absorption peak at 285 nm, the spectrum of DHA showed a smaller peak at 230 nm. A 4 ug standard zone gave a scan area about 50% larger at 285 nm compared to 235 nm, so the former was used for analysis of samples. To test whether better densitometric results could be obtained by scanning UV absorption rather than fluorescence quenching, a 4 ug standard of DHA was developed on a silica gel plate without fluorescent indicator and scanned at its absorption maximum, 307 nm

(3). The scan area the non-fluorescent plate was only ca. 10% greater than for quenching at 285 nm, and the calibration curve did not have significantly greater linearity. It was concluded that the convenience of being able to view the spot against the fluorescent plate background outweighed the minimal sensitivity advantage of direct scanning of UV absorption.

Calibration curves between 125-2000 ng for sorbic acid and 1-16 ug for benzoic acid and DHA had an average linear correlation coefficients for 45 trials of 0.95, with a range of 0.91 to 0.99. This strong linear correlation allowed quantification to be carried out reliably by comparing the sample area with the area of a closely matching single standard within the linear region. Considering the above calibration ranges and the ability to precisely apply 0.5 to 16 ul of samples, the quantification limits of the method extended from 7.8 to 4000 ppm for sorbic acid and 62.5 to 32,000 ppm for benzoic acid and DHA.

Table 1 shows recoveries obtained for samples of various juices and wines fortified with 50 to 300 ppm of the preservatives. All analyses were done on silica gel except those indicated with an asterisk, which were performed on a C-18 layer. Each analysis was run on a separate plate and was based on the average of duplicate sample and standard scan areas. As one measure of reproducibility, the percent difference between the scan areas of the duplicate samples and standards ranged from 0.30-5.4% for all analyses. Table 1 shows that recovery values ranged from 86 to 111% and averaged 98.0% for all analyses. These recoveries compare well with those reported for HPLC, which ranged from 90-104% for 250 and 50 ppm of sorbic and benzoic acid in juices (1) and 93-109% for 50 and 100 ppm of DHA in wines (2), and with the spectrophotometric results for sorbic acid in wine, which

TABLE 1

AVERAGE PERCENT RECOVERIES FROM FORTIFIED SAMPLES

Beverage	ppm	S	B	D
Apple juice	250	92.1	101	
		98.7	98.0	
		93.0*		
		96.8*		
	125		95.0	
			100	
	50	96.6		
		109		
Pineapple juice	250	106	97.5	
		105	102	
		111*		
	125		101	
			101	
	50	98.2		
		101		
Grapefruit juice	250	102		
		106		
	50	99.5		
		95.8		
		100		
Tomato juice	250	99.8		
		103		
		99.2		
	50	99.7		
		95.7		
Red wine	300	99.7		
		102		
	250			88.7
				86.0
				90.6
	125			90.2
				96.0
White wine	300	99.0		
		98.3		
Gold wine	250			92.8
				89.8
	125			94.7
				92.9
				90.2

S=sorbic acid, B=benzoic acid, D=dehydroacetic acid
* analysis performed on C-18 layer

ranged from 98-104% at concentrations of 200-400 ppm (2,4). They are also consistent with the generally acceptable 85-115% recovery range for ppm level chromatographic analyses performed to establish systematic error. All possible beverages were not tested with each preservative, but rather a reasonable assortment of samples and concentrations was tested in order to illustrate the levels of sensitivity, accuracy, and precision it was possible to achieve with the TLC method.

The method was used to analyze two commercial lemon flavored iced teas, one with added sugar and one containing NutraSweet, both of which listed an unspecified amount of potassium sorbate among their ingredients. The results of triplicate analyses were 223 ppm with a coefficient of variation (CV) of 1.8% for the sugar-containing tea and 102 ppm +/- 2.6% for the diet tea. The accuracy of these results was validated by adding 125 and 250 ppm of sorbic acid, respectively, to the teas and repeating the analyses as described above. The respective recoveries were 95.6 and 96.0%. The potassium sorbate was converted to sorbic acid during development with the acidic mobile phase.

Three canned sodas, a caffeine-free diet cola, a citrus soda, and a cream soda, containing unspecified concentrations of sodium benzoate were analyzed in triplicate, and the following respective concentrations (average +/- CV) were found: 137 ppm +/- 5.2%, 115 ppm +/- 0.87%, and 431 ppm +/- 3.9%. Many sodas contain sodium or potassium benzoate as a preservative, and our results indicate that the additive is present in a wide range of concentrations.

None of beverages used for recovery studies contained the analytes, as shown by the absence of zones with the same R_f value as the standards in the blank

chromatograms. In addition to the analyte zone, chromatograms of red and gold wine spiked at 250 and 125 ppm with DHA had an additional zone with an R_f value of 0.55. This unidentified zone did not interfere with scanning the DHA. For all samples, strongly sorbed, fluorescence-quenching compounds remained in the preadsorbent area and, for larger aliquots of some of the beverages, migrated a short distance beyond the interface into the silica gel area of the plate. Some of the sample chromatograms also had additional quenched zones at the solvent front.

In summary, the HPTLC method allows direct quantification of sorbic, benzoic, and dehydroacetic acid preservatives in a variety of beverages with accuracy and precision adequate for routine analytical use. The HPTLC procedure has several advantages over previously-published methods. The ability to spot multiple samples on a single plate provides higher sample throughput compared to sequential analysis by HPLC, and solvent usage is very low in the twin-trough chamber. The analysis of standards on the same plate under the same conditions as the samples eliminates the need for an internal standard, as used in HPLC (1). Direct injection of samples in HPLC (1,3) can leave irreversibly sorbed residues on top of the column that may affect column performance with later samples, while strongly sorbed residues from directly-spotted samples do not cause problems in TLC, where each plate is used only once. The HPTLC method does not require sample preparation by steam distillation, as do the spectrophotometric methods for sorbic acid in wine (2,4). Beverages could be analyzed for benzoic acid and DHA at lower concentration levels by HPLC than by our TLC method, i.e., 50 ppm (1) and 5 ppm (3), respectively.

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