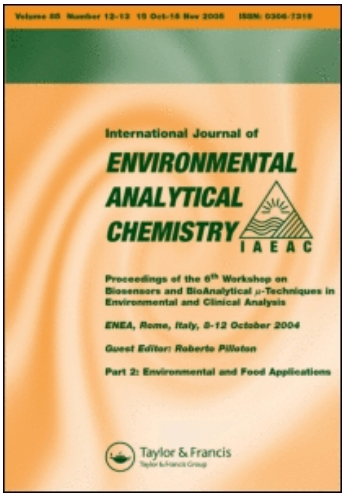

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Ion Chromatographic Determination of Cyanide Contamination in Fruit and Evaluation of a Colorimetric Test Kit

K. Chadha[a]; F. Lawrence[a]
[a] Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Ottawa, Ontario, Canada

To cite this Article Chadha, K. and Lawrence, F.(1991) 'Ion Chromatographic Determination of Cyanide Contamination in Fruit and Evaluation of a Colorimetric Test Kit', International Journal of Environmental Analytical Chemistry, 44: 3, 197 — 202
To link to this Article: DOI: 10.1080/03067319108027551
URL: http://dx.doi.org/10.1080/03067319108027551

Intern. J. Environ. Anal. Chem., Vol. 44, pp. 197–202
Reprints available directly from the publisher
Photocopying permitted by license only

ION CHROMATOGRAPHIC DETERMINATION OF CYANIDE CONTAMINATION IN FRUIT AND EVALUATION OF A COLORIMETRIC TEST KIT

K. CHADHA and F. LAWRENCE

Food Research Division, Bureau of Chemical Safety,
Food Directorate, Health Protection Branch,
Health and Welfare Canada,
Ottawa, Ontario, Canada. K1A 0L2.

(Received 28 February 1991)

Ion chromatography with electrochemical detection has been applied to the determination of cyanide in fruit spiked with potassium cyanide. The results were compared with those obtained by a cyanide test kit (Merkoquant 10044). There was good agreement in results at 45, 150 and 300 ppm levels of cyanide addition. The added cyanide was found to decrease on standing in the fruit matrix. Residual cyanide in fruit spiked at 500 ppm CN^- could be detected up to 3 days later by the kit method. However, with the HPLC method, detection was possible even after two to three weeks. Increase of sample pH seemed to improve recoveries by both methods

KEY WORDS: Ion chromatography, cyanide, fruit.

INTRODUCTION

In 1989, an incident in the USA involving cyanide contamination of grapes pointed out the need to determine toxic levels of cyanide in food matrices in a rapid quantitative manner.

A number of methods exist for the quantitative determination of cyanide. Spectrophotometry is one of the most commonly used techniques. Except for the analysis of relatively clean samples[1], a distillation step is usually employed to remove interfering substances and also to release cyanide from cyanogenic compounds present in some plants[2,3]. Ion chromatography has also been evaluated for cyanide but not applied to food type samples[4–6]. Some semiquantitative and qualitative test kits are commercially available and have been recently evaluated[7,8]. A recent method[9] for determination of endogenous cyanide has been published which involves distillation to isolate the cyanide before ion chromatography.

We report here an ion chromatographic method capable of determining cyanide present at ppm levels in fruit without distillation. In addition, we have compared the technique to a commercially available test kit, Merkoquant 10044.

EXPERIMENTAL

Reagents and materials

Degass Milli-Q water (Millipoe, Milford, MA) was used for making solutions. All chemicals were of reagent grade quality. The mobile phase was made with 1 litre of an aqueous solution containing 0.01 *M* H_3BO_3, 0.01 *M* NaOH and 0.001 *M* Na_2CO_3 which was passed through a 0.45 μm membrane filter and mixed with 0.25 g of ethylenediamine and 50 ml of methanol. A 0.25 ml volume of the stock spiking solution (15 mg KCN/ml) was sufficient to spike 5 g of fruit with 300 ppm CN^-. Dilutions of the stock solution were made to permit spiking at levels of 150 and 45 ppm CN^-. The cyanide test kit, Merkoquant 10044 (BDH Chemicals), was evaluated for rapid determination of cyanide and used exactly as instructed. Fresh fruit and juices were purchased locally.

Liquid chromatograph

The HPLC system consisted of a Beckman 112 Solvent Delivery Module, a Model 420 Controller and a Model 340 Organiser with a 20 μl loop. A Bioanalytical Systems LC-4B amperometric detector with a Ag working electrode at 0.0 V and Ag/AgCl reference electrode was used for detection. A Spectra Physics 4270 integrator was used to compute peak areas. Separations were carried out on a Waters IC Pak anion column with a Hamilton PRP X-100 guard column at ambient temperature (*ca* 20°C). The mobile phase flow was maintained at 1 ml/min.

Sample preparation

a) HPLC method—5 g of chopped fruit were weighed into a porcelain mortar and mashed to a smooth paste with a pestle. Sufficient 1*N* NaOH was added so that the final sample solution had a pH between 11 and 11.5. The sample was mixed well, made to 200 ml with water and filtered through Whatman #1 filter paper. For recovery studies, 0.25 ml of the spiking solution was added after NaOH addition and the procedure continued as above. For stability studies, samples were spiked, mixed and permitted to sit for various times before the addition of NaOH and HPLC analysis.

b) Kit method—Proceeded exactly as above without the addition of NaOH.

Determination

a) A3–4 ml volume of clear filtrate was passed through a 0.45 μm filter and an aliquot of the filtrate injected into the HPLC system. Cyanide was determined by comparing the peak areas of the sample with 0.25 ml of appropriate spiking solution added to 5 ml of water and taken through the same procedure as the fruit sample. For the kit method, 5 ml of clear filtrate were treated as described in kit. Cyanide was determined by comparing the results with 0.25 ml of appropriate spiking solution taken through the same procedure.

RESULTS AND DISCUSSION

Ion chromatographic separation of cyanide was first tried with 7.5 m*M* Na_2HPO_4 as an eluent and a glassy carbon working electrode at 0.8 V. However, the cyanide peak tailed very badly. The addition of 10% CH_3CN to the eluent did not improve the chromatography, nor did the use of 2 m*M* phthalic acid or borate buffer as eluents. Substitution of the Ag electrode for glassy carbon and inclusion of ethylenediamine in the eluent resulted in the best conditions we could obtain. The peak tailing was reduced but not eliminated. However, the selectivity of the system was such that high efficiencies were not essential. A 15 cm. Hamilton PRP X-100 column gave acceptable separation but with a longer retention time than the Waters column. Raising the column temperature[9] to 40°C resulted in only a small increase in peak height. Therefore, a column heater was not used for routine work.

Figure 1 shows typical chromatograms for a 45 ppm cyanide standard, a blank peach extract and an extract of peaches spiked with 45 ppm cyanide. The selectivity and sensitivity of the amperometric detector with the Ag electrode at 0.0 V enabled detection of added cyanide in grapes, peaches, tomatoes, plums, bananas, and grape juice. No cyanide was detected in any of the unspiked samples above the detection limit of about 50 ppb. A very small peak due to ascorbic acid was observed in some chromatograms just in front of the cyanide peak (ca 1.8 min), but this did not affect the results.

Table 1 compares recoveries of cyanide obtained from various samples using ion chromatography and the test kit method. Recoveries were very good for both methods at spiking levels of 150 and 300 ppm. The HPLC average was 94% compared to the test kit average of 88%. However, at the 45 ppm spiking level the test kit method gave an average of 69% compared to 89% by HPLC. The difference

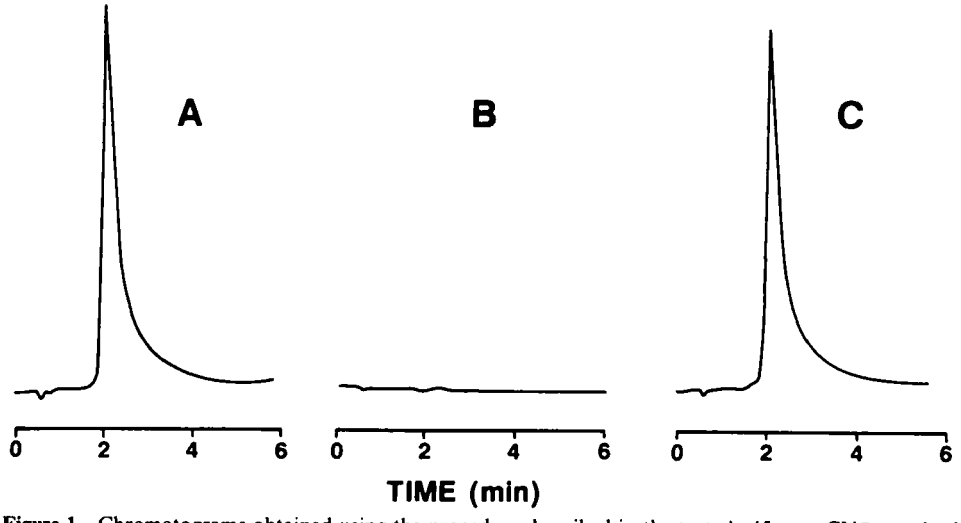

Figure 1 Chromatograms obtained using the procedure described in the text. A. 45 ppm CN^- standard B. Unspiked peaches C. Peaches spiked with 45 ppm CN^-

Table 1 Recovery of cyanide from selected fruit and vegetables.

Sample	*Cyanide spiking level (ppm)*	*Recovery*	
		HPLC	*KIT*
White grape juice (bottled)	45	95	72
	150	88	91
	300	98	100
Peaches	45	100	75
	150	100	91
	300	92	93
Tomatoes	45	78	69
	150	94	91
	300	99	84
Grapes	45	92	69
	150	96	87
	300	86	82
Banana	45	74	56
	150	91	88
	300	99	80
Plums	45	95	75
	150	96	83
	300	96	84

at this level may be due to the extraction conditions and the detection method employed. When no adjustment of pH (to 11) was carried out, the HPLC recoveries were erratic (sometimes less than 50%). The amperometric detector measures the anion, CN^- and the pK for HCN is 9.31, thus the pH of the solution had to be 11 or more to ensure the presence of cyanide as the CN^- species. This was not a requirement for the kit method, however, since the kit contains a buffer to adjust the sample pH to between 6 and 7 for the colorimetric reaction. The detection limits for cyanide were estimated to be 50 ppb by HPLC and 0.5 ppm by the kit method. The coefficient of variation of the chromatographic and the test kit method at a cyanide level of 300 ppm was 1.0% and 8.3% respectively.

Both methods performed well when sample extractions were carried out immediately after spiking. However, some spiked samples showed a rapid disappearance of cyanide if left at room temperature for even ten minutes before extraction and analysis. Figure 2 shows a plot of time before extraction versus concentration of cyanide found by HPLC of a plum homogenate spiked with 500 ppm of cyanide. As can be seen, the cyanide level dropped to 400 ppm even during the short time required to spike and make the first extraction. Within 2 hours, 50% of the cyanide had disappeared. The rate of decrease levelled off after approximately 3 days and the cyanide content remained stable at about 8% of the starting concentration for at least the next four days. Cyanide was detectable by HPLC in the homogenate even after 3 weeks. With the kit method, it was generally observed that cyanide could not

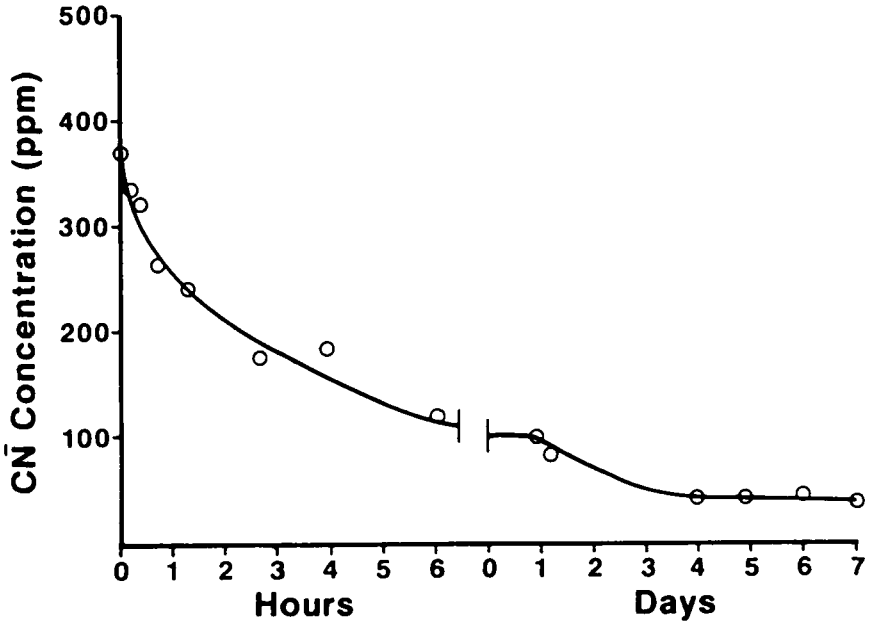

Figure 2 Recovery of cyanide with time from plums spiked with 500 ppm CN^-. Determined by HPLC.

be detected 2–3 days after spiking although by the HPLC method the cyanide was in the range of 50–100 ppm (much above the minimum detection limit of the kit method). When an aliquot of this solution was made to pH 11, then re-tested by the kit method, the response for cyanide was positive. It is believed that some cyanide bound to the matrix is not released under the pH conditions of the kit method but is released with the pH 11 extraction employed for ion chromatography.

The effect of raising the pH of the sample by addition of an alkali prior to determination was studied in detail using apple homogenate spiked at 500 ppm CN^-. The analysis was carried out at 0, 1, 2, 3, 4, 5, ,24 and 48 hours by both methods. Table 2 gives the results as per cent of total cyanide remaining by the HPLC and kit methods. The HPLC determinations were made both as described above and without the use of alkali. The kit determinations were made first as given in the kit, then with the addition of alkali and lastly with the addition-neutralization of alkali before the colorimetric determination.

The HPLC results, by the present method are seen to be slightly higher than the HPLC results without alkali at least for first few hours. Even if no alkali was used before determination, the high pH of the mobile phase will make the two HPLC results closer. The trend at 24 and 48 hours seems to be reversed. The kit results with the addition of alkali are significantly higher than those with the kit procedure (no alkali) where the cyanide has come down to just 1% in 48 hours. The alkali addition-neutralization results are similar with alkali addition—except for the 0, 4 and the 8 hours results. The differences were probably due to possible error in

Table 2 Effect of extract pH on cyanide recovery by HPLC and the kit methods.

	Cyanide remaining (%)				
Time (hr)	*HPLC*	*HPLC (no alkali)*	*Kit*	*Kit (alkali)*	*Kit (alkali-neutralized)*
0	69	55	54	93	60
1	55	49	38	83	74
2	51	44	36	67	74
3	45	37	32	60	65
4	45	42	26	59	36
5	40	36	12	52	54
24	25	31	6	36	37
48	14	21	1	19	11

neutralization as it was done with pH paper. It is apparent from the foregoing that the kit results can be lower and might even show a false negative for low levels of cyanide in the fruit matrix. In case of HPLC determinations the addition of alkali before spiking or determination only increased the initial results.

The HPLC and the kit methods were compared to the AOAC acid distillation procedure[10] using HPLC for the detection of cyanide in the distillate. A sample of apple homogenate was spiked with 500 ppm of cyanide, allowed to stand for 4 hours and analysed by the three methods. The amount of cyanide remaining was found to be 175, 157 and 136 ppm respectively by the distillation, ion chromatography and kit methods. The results by the three methods are approximately equivalent. The amount of cyanide undetected by all three methods was significantly large. Even distillation could not release the added cyanide which disappeared on standing. It seems that most of the cyanide is irreversibly bound or changed chemically. Preliminary work has indicated that the rate of CN^- disappearance is related to pH of the fruit matrix[11].

References

1. S. Upadhyay and V. K. Gupta, *Analyst*, **109**, 1619 (1984).
2. D. H. Honig, M. E. Hockridge, R. M. Gould and J. J. Rackis, *J. Agric. Food Chem.* **31**, 272 (1983).
3. C. H. P. van Eeden and A. W. J. de Jong, *Z. Lebensm Unters Forsch*, **181**, 412 (1985).
4. M. Nonomura and T. Hobo, *J. Chromatogr*, **465**, 395 (1989).
5. R. D. Rocklin and E. L. Johnson, *Anal. Chem.* **55**, 4 (1983).
6. A. M. Bond, I. D. Heritage and G. G. Wallace, *Anal. Chem.* **54**, 582 (1982).
7. US Food and Drug Administration Laboratory Information Bulletin No. 3352 "Evaluation of Cyantesmo Paper for Quantitative Detection of Exogenous Cyanide in Fruits" (Washington, DC, 1989).
8. Y. Kawamura, S. Uchiyama and Y. Saito, *Shokuhin Eiseigaku Zasshi*, **31**, 189 (1990).
9. Y. Kawamura, S. Uchiyama and Y. Saito, *Shokuhin Eiseigaku Zasshi*, **31**, 277 (1990).
10. Association of Official Analytical Chemists Official Methods of Analysis, 15th. Edition **Vol. 2**, 703 (1990).
11. R. K. Chadha, F. Bryce, J. F. Lawrence and H. B. S. Conacher and D. Arnold, accepted (1991) for publication in Fd. Chem. Toxic.