Journal of Chromatography, 83 (1973) 455–460

CHROM. 6784

APPLICATION OF LIQUID CHROMATOGRAPHY IN FOOD ANALYSIS

T. VAN DE WEERDHOF, M. L. WIERSUM and H. REISSENWEBER
Unilever Research Duiven, Zevenaar (The Netherlands)

SUMMARY

The usefulness of liquid chromatography is illustrated by the routine analysis of vitamin A, β-carotene, thiamine and riboflavine in foods in which two slightly different automated injection systems are used. The liquid chromatographic apparatus is constructed of separate component parts. The individual parts, including the two injection systems, are briefly discussed.

It is possible to carry out twenty-five vitamin A and β-carotene analyses or twenty-five thiamine and riboflavine analyses per day with a standard deviation of about 3%.

INTRODUCTION

The use of liquid chromatography (LC) in food analysis is of increasing interest, although LC is not yet competitive with gas chromatography in routine analysis. To illustrate the usefulness of LC, we describe in this paper the analysis of vitamin A, β-carotene, thiamine and riboflavine in food products. The generally accepted methods for the analysis of these vitamins are spectrometry, colorimetry or microbiological procedures[1,2]. The use of LC decreases the time required for an analysis. This time is reduced even more when the chromatographic analysis is automated. We therefore constructed two slightly different automatic injection systems, which enable us to operate the liquid chromatograph 24 h a day. Besides the above automated applications, we use LC for the analysis of several other components in food products.

APPARATUS

The LC apparatus was assembled from separate parts.

The pumps were either Milton Roy pulsating plunger pumps or diaphragm pumps from Orlita (Type DMP 1515) or from Whitey (Type LP 10). The Milton Roy pump cannot be used in every solvent system because the plunger is sealed with Viton or silicone O-rings. The diaphragm pumps are more reliable.

Next to the pumps a damping system is needed in order to smooth the flow fluctuations of the solvent. For this purpose, we used a damper developed by Deininger and Halász[3], which we found to be an excellent pressure control unit that functioned equally well as a relief valve and a flow programmer. The authors[3] mentioned

the use of three pumping heads to feed the damper, but we used one pumping head without losing a high degree of pressure control (1 % or better).

For the chromatographic columns, we prefer those made of glass, surrounded by a metal mantle that can also be used as a water-jacket[4]. The columns can be used at pressures up to 100 atm. When higher pressures are needed, metal columns must be used. In addition to refractive index and UV detectors we used fluorimetric detectors for the measurement of vitamin A, thiamine and riboflavine; β-carotene was detected colorimetrically.

For the fluorimetric detection, we used a self-constructed flow cell (Fig. 1), which was designed to fit in a Vitatron photometer (Vitatron, Dieren, The Netherlands). The internal volume of the cell was about 100 μl, which is small enough for most of the routine analyses. The self-constructed cell was placed in a Baird Atomic fluorimeter, for with this cell the sensitivity of the meter was about 10 times higher than with the original flow cell. When a fluorimeter is used equipped with monochromators such as the Baird Atomic fluorimeter, the width of the cell window through which the excitation light enters is, in fact, the slit width in use.

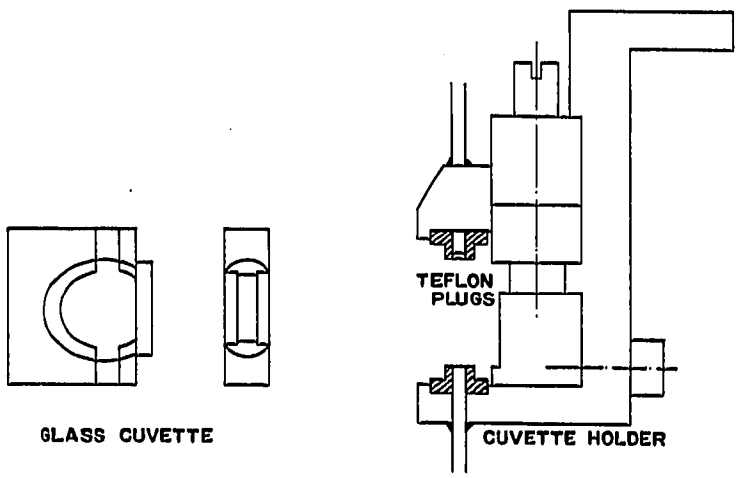


Fig. 1. Fluorimetric flow cell.

We constructed an automatic injector system, shown schematically in Fig. 2. The sample is injected on to the chromatographic column via a pneumatically driven Valco injection valve (Valco Instruments, Houston, Texas, U.S.A.). The volume of an external loop determined the volume of the sample to be injected. In order to fill this loop, one end was connected via a needle to the sample cup and the other end to a pneumatically driven syringe or a peristaltic pump which sucked the sample from the cup into the loop. To prevent contamination by a previous sample, the loop was first flushed with about five times the loop volume of the next sample.

When two valves were connected in series, the system could be used for injection on to two different columns. In this case, the time interval between two injections was determined by the longest time of the analysis.

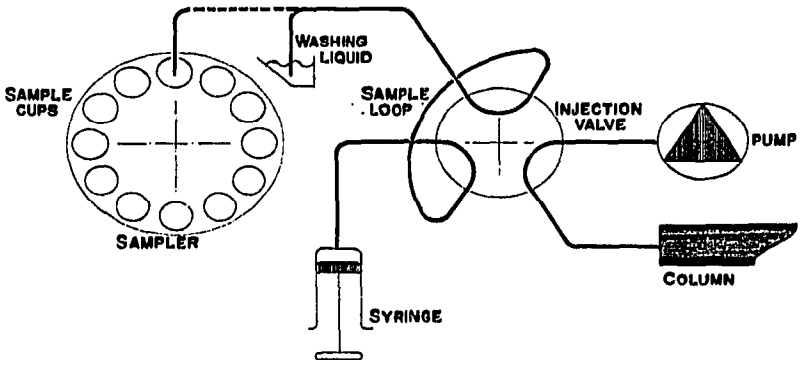


Fig. 2. Injection system.

PROCEDURE

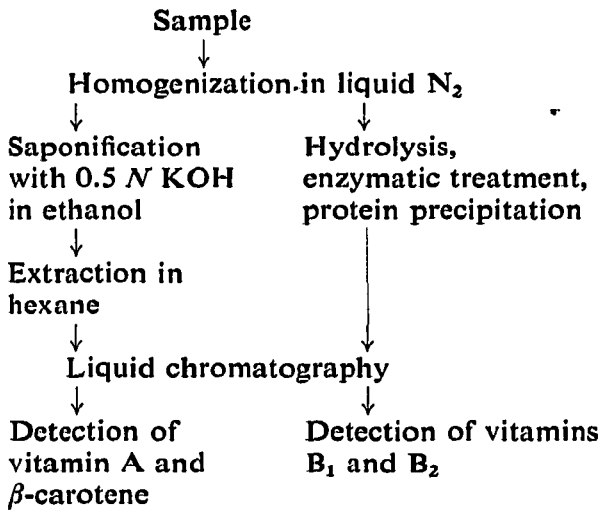
With the chromatographic analysis being automated, the time needed for the pre-treatment of the sample is the most important factor affecting the total time in vitamin analysis.

Sample pre-treatment

Vitamin A and β-carotene. For vitamin A and β-carotene, the procedure is as follows. The sample (*e.g.*, potatoes, meat, vegetables) is frozen in liquid nitrogen and homogenized in a Waring blender. 2.5 g of this sample are weighed in a 250-ml round-bottomed flask and 50 ml of 0.5 *N* KOH in ethanol and 1 ml of 10% Na_2SO_3 solution are added. After refluxing under nitrogen for 30 min, the mixture is cooled in ice, 100 ml of hexane are added and the mixture is transfered into a separating funnel containing 50 ml of 1 *N* KOH solution and shaken for 10 sec. After separation of the layers, the water layer is discarded. The hexane layer is washed with 50 ml of 1 *N* KOH solution and subsequently three times with 50 ml of water, and dried with Na_2SO_4. The hexane is now ready for injection on to the chromatograpihc column.

Thiamine and riboflavine. Thiamine and riboflavine can be present as such but also as phosphoric esters. In order to hydrolyse the esters and to set free the vitamins, the sample (*e.g.*, potatoes, meat, vegetables) is treated with acid and enzymes. A 2.5-g amount of the sample is placed in a centrifuge tube, and after addition of 10 ml of 0.25 *N* sulphuric acid, the tube is closed with an aluminium foil lid and heated for 30 min at 120°. After cooling the mixture to ambient temperature, 1.5 ml of acetate-acctic acid buffer (160 g of NaOH and 270 g of acetic acid per litre) is added and, after mixing, also 1 ml of takadiastase suspension (100 μg/ml) (Serva, Heidelberg, G.F.R.). This mixture is incubated for 15 min at 40°. Subsequently, 1 ml of a papain suspension (100 mg/ml) (Merck) is added and the mixture is incubated for a further 45 min. In order to precipitate the proteins, 2 ml of 45% trichloroacetic acid solution are added. After swirling, the sample is heated for 5 min at 50–60°. The sample is weighed and centrifuged, and the clear aqueous layer is ready to be used for the chromatographic separation.

The above working-up procedures can be summarized schematically as follows:



Liquid chromatography

The LC conditions for the analysis of the vitamins mentioned are given in Table I.

The fluorimetric detections are carried out with the self-constructed flow cell in a Vitatron photometer. Vitamin A and riboflavine can be measured as such by their native fluorescence. Thiamine must be oxidized to thiochrome, which is carried out after the chromatographic separation by mixing the effluent with an alkaline potassium hexacyanoferrate(III) solution (0.6 ml/min). This solution contains 10 ml of a 1% $K_3Fe(CN)_6$ solution, 240 ml of a 15% NaOH solution and 250 ml of water.

TABLE I
LC CONDITIONS FOR VITAMIN ANALYSES IN FOOD PRODUCTS

Vitamin	*Column*				*Mobile phase*	*Detection*		
	Length (cm)	*I.D. (cm)*	*Adsorbent*	*Particle diameter (μm)*		*Fluorimetric*		*Colorimetric (nm)*
						Excitation wavelength (nm)	*Emission wavelength (nm)*	
Vitamin A	50	0.3	Al_2O_3 + 5% H_2O	18–30	3% ethanol in benzene	325	514	—
β-Carotene	50	0.3	Al_2O_3 + 5% H_2O	18–30	1% ethyl acetate in hexane	—	—	460
Thiamine	25	0.3	silica gel	20–30	0.1 *M* phosphate buffer, pH 6.8, + 10% ethanol	362*	464*	—
Riboflavine	50	0.3	silica gel	20–30	0.1 *M* acetate buffer, pH 4.8	432	545	—

* After oxidation to thiochrome.

RESULTS AND DISCUSSION

The chromatograms of the vitamins in several food products are shown in Fig. 3. To determine the vitamins quantitatively, the peak heights are compared with standard peaks, a standard solution being injected after every fifth sample. The standard solution has to be injected so frequently because the samples, especially those which contain thiamine and riboflavine, are so complex that they contain components which can contaminate the column or change the permeability of the column. This results, in nearly all instances, is an increase in the analysis time and consequently in a change of peak height. If the time of analysis is increased by more than about 30%, the column is changed, which may be necessary after 1 week or 1 month. For the water-soluble vitamins, it is sometimes necessary to change the column after a few days.

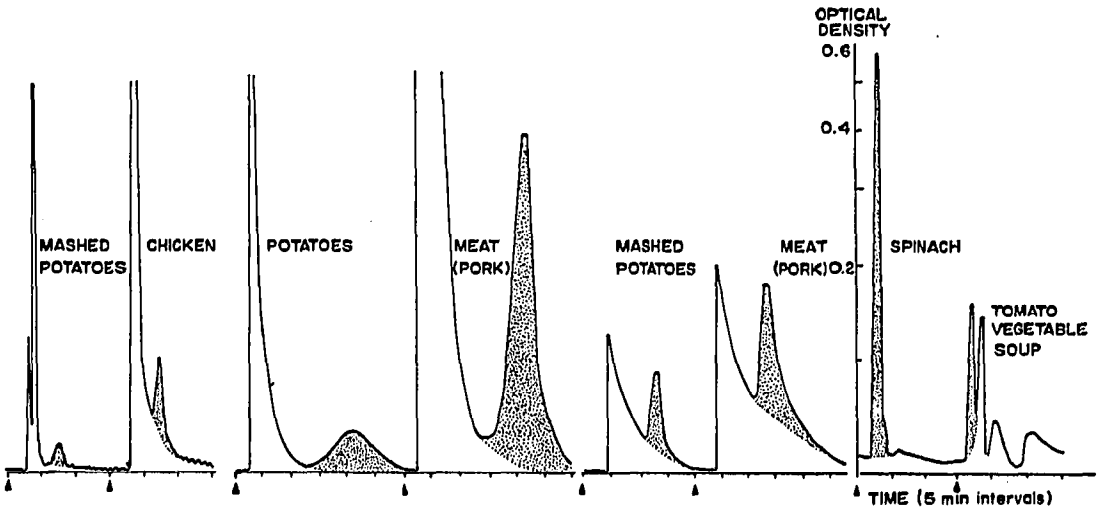


Fig. 3. Eight liquid chromatograms of vitamins in food products. From left to right: 1, 2, vitamin A; 3, 4, thiamine; 5, 6, riboflavine; 7, 8, β-carotene. Chromatograms 1–6 have linear scales.

The detection limits of these vitamins are 5 μg for β-carotene and 2 μg for the others. The concentration limit in the starting material that can be measured by using the working-up procedure described is 0.03 mg per 100 g for β-carotene and 0.01 mg per 100 g for the others. When concentration stages are included, the amount of vitamins that can be detected in the starting material can be much lower. We were, however, not interested in food products which contribute only a small amount of vitamins to the daily intake. The recoveries (when 1–4 μg of vitamin A, thiamine and riboflavine and 25 μg of β-carotene are added to 2.5 g of the starting material) are between 90 and 100%.

The main advantage of LC in food analysis is demonstrated by the speed of analysis of vitamins. Including sample pre-treatment, one person can perform 25 vitamin A and β-carotene analyses or 25 thiamine and riboflavine analyses per day.

In our laboratory we have also used the technique with success for the final

separation and measurement of 3,4-benzpyrene in sausages, aflatoxins in groundnuts and flavour components in natural products.

REFERENCES

1 R. Strohecker and H. M. Henning, *Vitamin-Bestimmungen*, E. Merck AG, Darmstadt, 1963
2 F. J. Mulder, *Dissertation*, University of Amsterdam, 1957.
3 G. Deininger and I. Halász, *J. Chromatogr.*, 60 (1971) 65.
4 J. F. K. Huber, *J. Chromatogr. Sci.*, 7 (1969) 85.