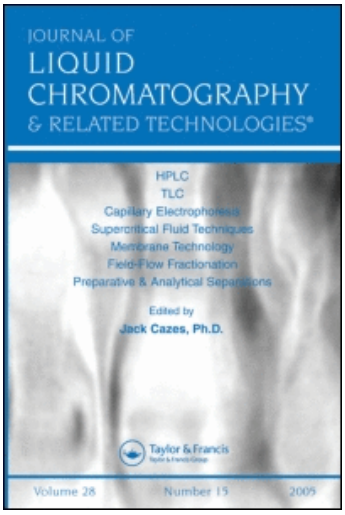


## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Separation and Determination of Dyes by Ion-Pair Chromatography

J. J. Berzas-Nevado[a]; C. Guiberteau-Cabanillas[a]; A. M. Contento-Salcedo[a]
[a] Department of Analytical Chemistry and Food Technology, University of Castilla-La Mancha, Ciudad Real, Spain

**To cite this Article** Berzas-Nevado, J. J. , Guiberteau-Cabanillas, C. and Contento-Salcedo, A. M.(1997) 'Separation and Determination of Dyes by Ion-Pair Chromatography', Journal of Liquid Chromatography & Related Technologies, 20: 18, 3073 — 3088

**To link to this Article: DOI:** 10.1080/10826079708006582

**URL:** http://dx.doi.org/10.1080/10826079708006582

J. LIQ. CHROM. & REL. TECHNOL., 20(18), 3073–3088 (1997)

# SEPARATION AND DETERMINATION OF DYES BY ION-PAIR CHROMATOGRAPHY

J. J. Berzas-Nevado, C. Guiberteau-Cabanillas,
A. M. Contento-Salcedo

Department of Analytical Chemistry and Food Technology
University of Castilla-La Mancha
13071 Ciudad Real, Spain.



## ABSTRACT

A reverse phase ion-pair high performance liquid chromatographic method is presented to determine six food dyes (E-110, E-120, E-122, E-123, E-124 and E-127). The tetra-butylammonium ion (TBA) is used as counter-ion in the mobile phase. The separation was accomplished with a Nova-pack $C_{18}$ column using methanol -$NaH_2PO_4$/$Na_2HPO_4$ pH=7 buffer solution 0.1M containing TBA 0.05 M as mobile phase. The chromatograms were monotorized by measuring the absorbance at 520 nm. The influence of methanol content, counter ion concentration, pH and flow-rate of the mobile phase were investigated. In the chromatographic conditions selected, the total chromatogram was obtained in seven minutes. The calibration graphs were established by measuring the peak area in the chromatograms. Determination limits ranging from 1 to 7.8 ng were obtained. Samples containing the six dyes in different proportions were analyzed through the proposed method, obtaining good recoveries in all cases. Finally, the method was successfully applied to the analysis of dyes in several commercial products.

## INTRODUCTION

Synthetic colors are added to foods to replace natural color lost in processing, to reduce batch to batch variation, and to produce products with consumer appeal where no natural color exists. In recent years, food additives have increasingly come under investigation for evaluation of their safety in use.

Toxicological investigation of food colors is underway in many countries.[1,2] These toxicological data result in repeated revisions of a number of permitted food dyes.

In the European Community, a Scientific Committee has reviewed the safety in the use of all compounds proposed for inclusion in a Community List of coloring matters, authorized for use in foodstuffs.[3] Special attention was paid to the sulfonated azodyes. Systematic studies on chronic toxicity have led pharmacologists and nutritionists to define acceptable daily intakes (ADI) for each of the permitted dyes.



The dyes studied in this paper contain sulfonic or carboxylic acid function and are present as anions over a large pH range. In a reverse phase HPLC system, anionic substances have very short retention times. An ion-pair chromatographic system would be a way of achieving adequate retention. When a hydrophobic ion of opposite charge (counter ion) is added, the retention of the ionized substance in a nonpolar stationary phase is enhanced. Because the ion-pair formed is more hydrophobic than the ionized molecule, it will be retained for a longer time inside the column.

The main problem in the quantitative determination of dyes in food is at the extraction step. In this way, the colors have been isolated from food by dyeing the color onto wool,[4] by polyamide columns,[5] ion-exchange resins,[6,7] and by liquid extraction using an ion-pair reagent.[8,9] Several other methods involving the use of $C_{18}$[10,11] or amine quaternary[12] columns have been described more recently.

High performance liquid chromatography (HPLC) has became a popular technique to determine synthetic food dyes. For instance, although ion exchange[13] and reverse phase HPLC[14,16] has both been used, it is the reverse phase ion-pair HPLC[17-20] that has been found particularly useful for the separation and detection of several dyes. In the ion-pair chromatography of anionic substances, several counter ions such as cetrimide,[21,22] a cationic detergent, and tetra-alkylammonium ion[23-25] have been used.

In this report, a reverse phase ion-pair HPLC method is proposed for the separation and quantification of six food colors. Tetrabutylamoniun ion (TBA) was chosen as counter-ion in the mobile phase. The optimal conditions were selected and the method was applied to the quantification of these substances in commercial products.

## EXPERIMENTAL

### Apparatus

The chomatographic system consisted of Waters variable wavelength UV-Vis detector model 486, a quaternary gradient pump Waters series 35 equipped with a solvent programmer, a Rheodyne Model 7125 injector with a 20 µL sample loop, and a NEC 386/25 computer fitted with Water Baseline software were used for all the measurement and treatment of data.

The detection wavelength was 520 nm. The analytical column was a Nova-Pack C18 (3.9 mm x 150 mm's ID, partic'e size 3 µm).

The pH values were measured with a Crison, model 2001 with a combined glass electrode.



### Reagents

All solvents and reagents were of analytical grade unless indicated otherwise. Solutions were made with deionized water (Milli-Q quality).

- Dyes standards solutions: Pure samples of Amaranth (E-123), Ponceau 4R (E-124), Carmoisine (E-122), Sunset Yellow (E-110), and Erythrosine (E-127) were obtained from Aldrich Company and Carminic acid (E-120) from Sigma Company. The stock solutions (200 mg/L) were prepared by dissolving pure samples in deionized water, except E-127, that was dissolved in ethanol.

- Tetra-n-butylammonium hydroxide 40% aqueous solution (Sigma) diluted to 10% with deionized water. This solution was used as a TBA stock solution to prepare mobile phases.

- Buffers: Composition for 2 L and ionic strength 0.1 M: pH = 7.0 ; 5.780 g $NaH_2PO_4.H_2O$ , 9.380 g $Na_2HPO_4.2H_2O$. pH = 4.5 ; 0.9 mL 1M $H_3PO_4$ , 27.598 g $NaH_2PO_4.H_2O$. pH = 3.0; 32 mL 1M $H_3PO_4$, 27.598 g $NaH_2PO_4.H_2O$.

- Methanol was HPLC grade from Panreac.

## RESULTS AND DISCUSSION

Six food dyes were investigated in this paper: Amaranth (E-123), Ponceau 4R (E-124), Carmoisine (E-122), Sunset Yellow (E-110) and Erythrosine (E-127). As commented before, these compounds are anionic species in a large pH range and low retention times are observed in a reverse phase HPLC system. Retention and selectivity can be controlled by the formation of an ionic- pair, and by the selection of the type and concentration of the organic solvent in the mobile phase. Addition of inorganic electrolytes or adjustment of its pH can also be used to improve the separation.

In this work, we have used the ion-pair chromatography to study the separation of these dyes on a Nova-Pack C18 column with the chromatographic system above described.

The mobile phase consisted of methanol - phosphate buffer solution mixture containing tetra-n-buthylammonium ion (TBA) as the ion-pairing reagent.

The flow-rate was fixed at 1 mL/min unless indicated otherwise. Before use, the eluents were filtered through Millipore filter and dissolved gases were removed by purging with helium for 10 min. The wavelength selected to monotorize the chromatogram was 520 nm.

The influences of methanol percent, counter ion concentration and pH value in the mobile phase as well as the mobile phase flow-rate in the separation procedure will be discussed in the next sections.

### Effect of Methanol Content in the Mobile Phase on the Retention

In these experiments, the reservoirs of the solvent system were filled with the following two eluents:

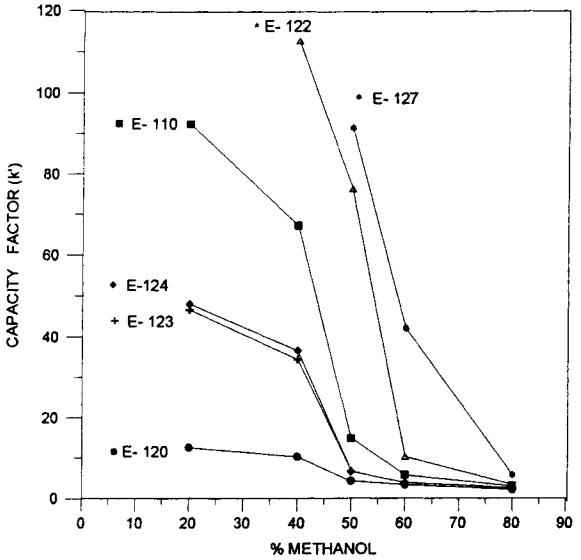


**Figure 1**. Influence of methanol percent in the mobile phase on capacity factor of various dyes using a reverse phase ion -pair HPLC system.

-Eluent A consisted of methanol (20%), phosphate buffer solution 0.1M pH=7 (80%) and TBA 0.005M.
-Eluent B consisted of methanol (80%), phosphate buffer solution 0.1M pH=7 (20%) and TBA 0.005M.

Both eluents were mixed and pumped to the column with the solvent programmer.

For this study 20 μL of each dye solution were injected individually into the column and chromatograms were obtained with a different methanol percentage in the mobile phase.

Figure 1 shows the influence of methanol percentage in the mobile phase on retention (capacity factor) of this group of dyes. Retention decreases with higher methanol concentrations in the mobile phase because the polarity of the eluent also decreases. Indeed, the retention times are spread over a wide range. E-110, E-122 y E-127 exhibit long retention times, and need high a methanol percentage to be eluated. However E-123, E-124 and E-120 are hardly retained with this methanol percentage. The very different retention properties of the dyes requires a methanol gradient elution system to achieve an adequate separation.

**Table 1**

**Chromatographic Parameters of a Group of Dyes***

| Dye | Retention Time RT | Capacity Factor k′ | Resolution $R_s$ |
|---|---|---|---|
| E-120 | 1.57 | 5.28 | ---- |
| E-123 | 3.33 | 12.32 | 4.63 |
| E-124 | 4.93 | 18.72 | 4.57 |
| E-110 | 5.48 | 20.92 | 2.29 |
| E-122 | 6.13 | 23.52 | 3.02 |
| E-127 | 7.67 | 29.68 | 4.74 |

* See text

$k' = (RT_1 - RT_0) / RT_0$

$R_s = 2(RT_2 - RT_1) / (W_1 + W_2)$

$RT_2$ and $RT_1$ : retention time of solutes 2 and 1 which are successively eluated. $RT_0$ : retention time of an unretained solute. $W_1$ and $W_2$ : peak width of solutes 1 and 2



**Gradient Elution Optimized**

Using the solvent programmer, it is possible to change the proportion of eluent A and B, and also the methanol percent, during the cromatography.

Several methanol gradients were assayed and a gradient profile consisting of a linear change from the initial eluent composition (60% eluent A , 40% eluent B) to the final composition (100% eluent B) was selected as follows:

$t_0$ (init.) 60% eluent A, 40% eluent B
$t_1$ (1 min.) 50% eluent A, 50% eluent B
$t_2$ (3 min.) 100% eluent B
$t_3$ (8 min.) 100% eluent B

Therefore, methanol concentration varied from 44% to 80%.

In Table 1 the retention time (RT) and capacity factor (k′) of each dye are summarized. In addition, the chromatographic resolution ($R_s$) between two adjacent peaks were calculated and are showed in the same table. The gradient profile selected permits an excelent separation, as can be ascertained from $R_s$,, in a short time, about eigth min.

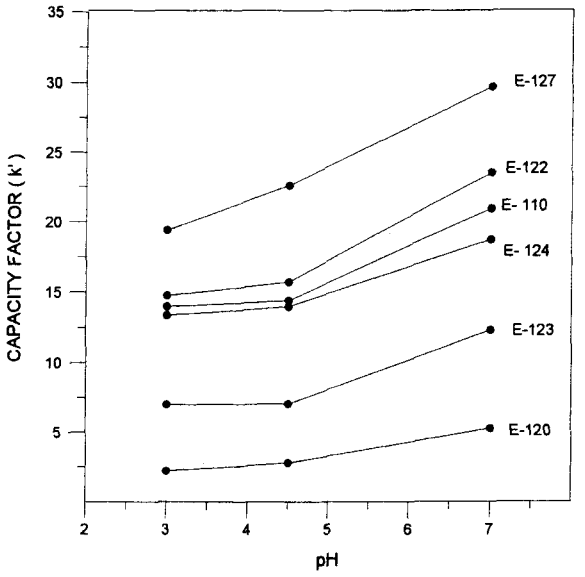


**Figure 2**. Influence of pH in the mobile phase on capacity factor of various dyes using a reverse phase ion -pair HPLC system.



## Effect of pH in the mobile phase on the retention

The influence of pH of the mobile phase on the retention propertes was studied. For this purpose, the pH value was fixed in the eluents A and B by using different phosphate buffer solutions. Chromatograms of a solution with all dyes were obtained with the gradient elution profile selected before. Figure 2 shows, graphically, the behaviour of this group of dyes at three pH values. As can be seen, the retention increases when pH also increases. A pH value of 7.0 was selected to ensure ionisation of dyes and, also, the ion pair formation with the TBA, without the eluents damaging the silica packing material. An $NaH_2PO_4/Na_2HPO_4$ solution of pH 7 (ionic strength 0.1) was used for buffering.

## Effect of counter ion concentration

With the aim to study the influence of counter-ion in the mobile phase on capacity ratio, the chromatograms were obtained, according to the gradient profile selected, with different TBA concentration in the eluents.

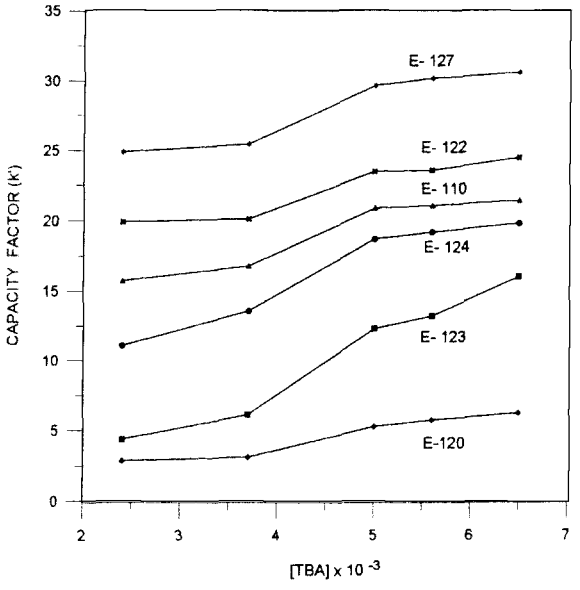


**Figure 3**. Influence of TBA concentration in the mobile phase on capacity factor of various dyes using a reverse phase ion -pair HPLC system.



As can be seen in Figure 3, the retention times for all dyes increase when the counter ion concentration increase. The chromatographic resolutions ($R_s$) were calculated in each chromatogram. A concentration of TBA 0.005 M was selected because it provided $R_s$ values higher than 1.5.

### Effect of flow-rate of the mobile phase

Finally, the influence of the flow- rate of the mobile phase was studied. The retention times and chromatographic resolution between peaks decrease when the flow-rate of mobile phase increase. A flow-rate of 1.5 mL/min was selected. In all cases satisfactory chromatographic resolution between peaks ($R_S$) were obtained.

## CHROMATOGRAPHIC PROCEDURE SELECTED

From the studies carried out before, we propose a chromatographic procedure to determine six food dyes, wich is described in this section.

Two eluents (eluents A and B) were mixed to form the mobile phase:
Eluent A: methanol (20% ), $NaH_2PO_4/Na_2HPO4$ buffer solution 0.1M pH=7 (80%) and TBA 0.005M.
Eluent B: methanol (80%), $NaH_2PO_4/Na_2HPO_4$ buffer solution 0.1M pH=7 (20%) and TBA 0.005M.

Before use, the eluents were filtered through Millipore filter and dissolved gases were removed by purging with helium for 10 min.

The proportions of eluent A and B were varied during chromatography by using the solvent programmer. The gradient profile consisted of a 1 min. linear change from 60% eluent A and 40% eluent B to 50% eluent A, 50% eluent B , then a second 2 min. linear change to the final composition (100% eluent B) and finally, a isocratic period (5min.) using the final composition. After chromatography, the mobile phase was returned to initial conditions using a 2 min. linear gradient.

The flow-rate of the mobile phase in the column was set at 1.5 mL/min. The column was conditioned by pumping initial eluent composition at least 5 min. before injection of the sample.

20 μL volume of a dye solution was injected in the chromatographic system. The gradient elution was initiated and absorbance monitorized at 520 nm. Duplicate chromatographic separation were made for all dye solutions. The chromatographic conditions optimized are summarized in Table 2 and the chromatogram corresponding of a standard of the six dyes is shown in Figure 4. From this chromatogram, it can be ascertained that the chromatographic procedure selected is excellent for the separation of the solutes.



## QUANTITATIVE DETERMINATION

### Calibration Graphs

The linearity of the assay was checked by running, in duplicate, a set of standards through the chromatographic procedure decribed above.

Two calibration graphs were obtained for each dye by plotting the peak area and the peak height versus the concentration. A good linearity was obtained for all dyes in the range studied: 8-40 mg/L for E- 120 and 2-10 mg/L for the rest of them.

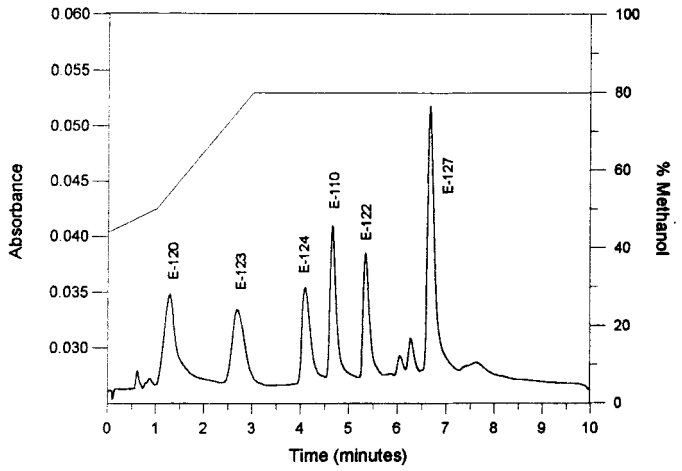


**Figure 4**. Chromatogram of dyes mixture obtained by the proposed method and the gradient profile selected (see text and Table 2).



## Table 2

## Chromatographic Conditions Selected

| | |
|---|---|
| **Column:** | **Nova-Pak C18** |
| **Mobile phase** | |
| Eluent A | methanol (20%), phosphate pH=7 buffers (80%), TBA (0.005 M) |
| Eluent B | methanol (80%), phosphate pH=7 buffers (20%), TBA (0.005 M) |
| **Gradient profile** | |
| | $t_0$ (initi) 60% eluent A, 40% eluent B |
| | $t_1$ (1 min.) 50% eluent A, 50% eluent B |
| | $t_2$ (3 min.) 100% eluent B |
| | $t_3$ (8 min.) 100% eluent B |
| **Flow-rate** | 1.5 mL/min |
| **Injection volume** | 20 μL |
| **Detection** | 520 nm |

**Table 3**

**Statistical Parameters**

| Dye | | Standard Deviation (mg/L) | Relative Standard Deviation | Determination Limit[1] (ng) |
|---|---|---|---|---|
| E-120 | Area | 0.853 | ± 8.29 | 7.8 |
| | Height | 1.351 | ± 12.85 | 52.0 |
| E-124 | Area | 0.029 | ± 1.87 | 1.0 |
| | Height | 0.053 | ± 3.55 | 16.6 |
| E-110 | Area | 0.071 | ± 4.22 | 1.4 |
| | Height | 0.037 | ± 2.43 | 15.6 |
| E-123 | Area | 0.036 | ± 2.21 | 7.6 |
| | Height | 0.036 | ± 2.26 | 17.0 |
| E-122 | Area | 0.039 | ± 2.32 | 7.6 |
| | Height | 0.023 | ± 1.28 | 26.0 |
| E-127 | Area | 0.060 | ± 3.61 | 5.6 |
| | Height | 0.038 | ± 2.20 | 14.0 |

[1] $C_Q = 10\ S_B / m$: $C_Q$ = determination limit; $S_B$ = standard deviation of blank; m = slope of calibration graph.



## Statistical

Precission of the assay was checked by performing nine replicate runs of mixtures of six dyes. The standard deviation and the relative standard deviation were calculated. The determination limits were also obtained for each dye.[26] In Table 3 the statistical data obtained are summarized.

## Analysis of synthetic mixtures

To test the validity of the proposed method, several synthetic mixtures containing all dyes in different proportions were prepared and analyzed using the chromatographic procedure described.

## Table 4

## Recoveries Obtained in the Analysis of Synthetic Mixtures Using the Peak Area

| Composition of Mixtures (mg/L) | | | | | | Recoveries (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C.A. | P | AN | AM | C | E | C.A | P | AN | AM | C | E |
| 20 | 9.6 | 3.2 | 3.2 | 9.6 | 3.2 | 95.92 | 97.75 | 105.5 | 96.00 | 101.75 | 94.00 |
| 32 | 6.4 | 6.4 | 6.4 | 4.8 | 4.8 | 100.68 | 97.60 | 99.75 | 101.00 | 95.75 | 95.25 |
| 24 | 4.8 | 9.6 | 9.6 | 3.2 | 9.6 | 101.86 | 91.25 | 100.40 | 101.00 | 98.50 | 99.00 |
| 16 | 3.2 | 4.8 | 4.8 | 6.4 | 6.4 | 103.77 | 87.75 | 96.50 | 104.60 | 103.75 | 98.00 |
| 8 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 111.17 | 87.75 | 93.37 | 94.62 | 97.75 | 92.75 |
| **Statistical Parameters*** | | | | | | | | | | | |
| | | | | | | X | 100.55 | 95.53 | 99.10 | 99.44 | 99.54 | 95.80 |
| | | | | | | S | 3.34 | 3.71 | 4.54 | 4.08 | 3.18 | 2.64 |
| | | | | | | $S_m$ | 1.67 | 2.14 | 2.03 | 1.82 | 1.42 | 1.18 |

C.A.: E-120, P: E-124, C: E-122, AN: E-110, AM: E-123, E: E-127/

* Values of recoveries higher than 110% or smaller than 90% have not been considered for calculating these parameters.



## Table 5

## Recoveries Obtained in the Analysis of Synthetic Mixtures Using the Peak Height

| Composition of Mixtures (mg/L) | | | | | | Recoveries (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A.C. | P | AN | AM | C | E | C.A | P | AN | AM | C | E |
| 32 | 9.6 | 9.6 | 4.8 | 6.4 | 3.2 | 95.51 | 81.25 | 88.25 | 82.50 | 92.55 | 91.50 |
| 24 | 6.4 | 4.8 | 6.4 | 4.6 | 1.6 | 106.16 | 83.40 | 81.00 | 85.75 | 89.00 | 90.25 |
| 16 | 4.8 | 6.4 | 1.6 | 3.2 | 4.6 | 81.26 | 70.75 | 85.45 | 78.50 | 91.75 | 93.50 |
| 20 | 3.2 | 1.6 | 9.6 | 4.8 | 4.8 | 112.02 | 68.75 | 81.50 | 90.60 | 91.75 | 92.36 |
| 8 | 1.6 | 4.8 | 3.2 | 9.6 | 6.4 | 142.62 | 67.50 | 83.25 | 85.52 | 96.00 | 93.25 |
| **Statistical Parameters*** | | | | | | | | | | | |
| | | | | | | X | 107.51 | 73.48 | 83.90 | 89.10 | 92.25 | 92.20 |
| | | | | | | S | 22.84 | 6.96 | 2.67 | 4.15 | 2.50 | 1.34 |
| | | | | | | $S_m$ | 10.21 | 3.11 | 1.19 | 1.86 | 1.12 | 0.60 |

C.A.: E-120, P: E-124, C: E-122, AN: E-110, AM: E-123, E: E-127/

* Values of recoveries higher than 110% or smaller than 90% have not been considered for calculating these parameters.

The results obtained using the area and height peak are summarized in Table 4 and 5 respectively. The recoveries (%) obtained show that the determination of all dyes are suitable, with best results when we used the peak area as an analytical signal of measurement.

**Analysis of commercial samples**

The method described was applied to analyze commercial products containing two or more of the dyes studied. Thus, we have analyzed three beverages (Bitter Kas, Bitter Kalty and grenadine), gelatin desserts, and syrup with strawberry flavor. The amount of dye in each sample was calculated by using the calibration graph established and by using the standard addition method.

The samples were prepared as follows:

A) Beverages (Bitter Kas, Bitter Kalty and grenadine).

1) Quantitative determination by direct preparation using the calibration graphs established: 5 mL of the sample was transferred to a 25- mL flask and diluted with deionized water to the mark.

2) Quantitative determination by standard addition: to 5mL of the beverage sample were added different amounts (2, 4, 6, 8 mg/L) of the dye to determine and proceed as before.

B) Gelatin and syrup.

1) Quantitative determination by direct preparation using the calibration graphs established: 1g product was diluted with 10 mL of deionized water in beaker over low heat with gentle swirling until dissolved. The solution was filtered and transferred to a 25-mL flask and diluted with deionized water to the mark.

2) Quantitative determination by standard addition: to 1g product were added different amounts (2, 4, 6, 8 mg/l) of dye to determine and proceed as before.

The chromatograms of all solutions were recorded according to the chromatographic procedure, and the peak area of each compound was obtained. The results obtained, using the calibration graph and the standard addition method, were very similar and are summarized in Table 6.

**Table 6**

**Analysis of Commercial Samples**

| | Bitter Kas | | Bitter Kalty | | | Grenadine | |
|---|---|---|---|---|---|---|---|
| | C(mg/L) | P(mg/L) | C(mg/L) | AM(mg/L) | AN(mg/L) | P(mg/L) | AM(mg/L) |
| Direct measurement | 34.3 ± 0.1 | 10.3 ± 0.2 | 16.7 ± 0.3 | 38.3 ± 0.4 | 6.9 ± 0.2 | 50.8 ± 0.3 | 29.5 ± 0.3 |
| Standard addition | 35.0 ± 0.1 | 9.9 ± 0.1 | 16.7 ± 0.3 | 39.9 ± 0.3 | 8.8 ± 0.2 | 50.4 ± 0.2 | 28.6 ± 0.2 |

| | Gelatine | | Syrup | |
|---|---|---|---|---|
| | AM(mg/Kg) | P(mg/Kg) | P(mg/Kg) | C(mg/Kg) |
| Direct measurement | 160.0 ± 0.4 | 275.0 ± 0.3 | 118.8 ± 0.1 | 146.2 ± 0.3 |
| Standard addition | 161.2 ± 0.3 | 278.7 ± 0.3 | 119.1 ± 0.2 | 143.6 ± 0.3 |



## CONCLUSIONS

The experimental results obtained in this paper, demonstrate that the chromatographic method proposed is sufficiently specific, sensitive, accurate, and rapid to analyze red and orange dyes, authorized for use in food in Spain, in commercial products.

## ACKNOWLEDGMENT

The authors gratefully acknowledge financial support from the " Dirección General de Investigación Científica y Técnica" (DGICYT) (Project No. PB 94-0743).

## REFERENCES

1. K. S. Khera, I. C. Munro, CRC Critical Rev. Toxicol., **6(2)**, 81-133 (1979).

2. K. Venkataraman, **Analytical Chemistry of Synthetic Dyes**, Wiley Interscience, New York, 1977.

3. Directiva 94/34 del Parlamento Europeo y del Consejo del 30 de Junio de 1994, Diario Oficial de las Comunidades Europeas, Nº L 273/13. 10/9/1994.

4. **Separation and Identification of Food Colors Permitted by the Coloring Matter in Food Regulation**, Assoc. of Public Analyst, 1957.

5. H. Egan, R. S. Kirk, R. Sawyer, **Pearson's Chemical Analysis of Food**, 1981

6. C. J. Graichen, Assoc. Off. Anal. Chem., **58 (2)**, 278 -82 (1975).

7. N. P. Boley, N. G. Bunton, N. T. Crosby, A. E. Johnson, P. Roper, L. Somers, Analyst, **109**, 589-599 (1980).

8. M. Puttermans, L. Dryon, L. Massart, Anal. Chim. Acta., **113**, 307, 314 (1980).

9. M. Puttermans, L. Dryon, L. Massart, J. Assoc. Off. Anal. Chem., **65(3)**, 737 -44 (1982).

10. M. L.Young, J. Assoc. Off. Anal. Chem., **67, (55)**, 1022-24 (1984).

11. J. L. Love, New Zeland Journal of Science, **27**, 113-16 (1984).

12. T. Hayash, Y. Fuyaka, H. Tanaka, M. Kunimatsu, I. Hotta, Y. Ikai, H. Oka, J. Hayakawa, R. Suzuki, Shokuhin Eiseigaku Zasshi, **34 (5)**, 398-403 (1993).

13. V. Rizova, T. Stafilov, Anal. Letter, **28 (7)**, 1305-16 (1995).

14. M. Nishizawa, T. Chonan, Y. Hori, Hokkaidoritsu Eisei Kenkyushoho, **35**, 7-11 (1985).

15. M. Li, Y. Hu, G. Shao, Sepu., **6 (1)**, 44-6 (1988).

16. F. Wu, P. Zhang, Sepu., **10 (5)**, 311 - 312 (1992).

17. F. E. Lancaster, J. F. Lawrence, Trace Subst. Envirom. Health, **15**, 303- 15 (1981).

18. F. E. Lancaster, J. F. Lawrence, J.Assoc.Off.Anal. Chem., **65 (6)**, 1305- 10 (1982).

19. F. E. Lancaster, J. F. Lawrence, J.Assoc.Off.Anal. Chem., **66 (6)**, 1424- 8 (1983).

20. W. Yue, Y. Zhu, C. Jiang, G. Yang, Fenxi Huaxue, **20 (7)**, 797-9 (1992).

21. J. Chudy, N. T. Crosby, I. Patel, J. Chromatogr., **154**, 306 -312 (1978).

22. M. Porthault, C.Gonnet, J. L. Rocca, Labo-Pharma-Probl. Tech., **26 (277)**, 498 - 502 (1978).

23. S. P. Sood, L. E. Sartori, D. P. Wittaner, G. M. Haney, Anal Chem., **48**, 796-799 (1976).

24. S. Eksborg, P. O. Lagerstrom, R. Modin, G. J. Schill, Chromatogr., **83**, 99 -110 (1973).

25. J. H. Knox, J. J. Jurand, Chromatogr., **149**, 297 -312 (1976).

26. J. A. Glaser, D. L. Foerst, G. D. Mckee, S. A. Quave, W. L. Budle, Environmental Sci. and Tecnol., **15**, 1427 (1981).

Received: July 25, 1996
Accepted: March 27, 1997
Manuscript 4223