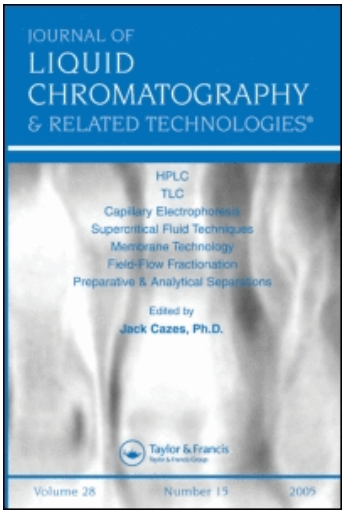

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SEPARATION OF COLOR PIGMENTS OF CHILI POWDER ON NARROW-BORE HPLC COLUMNS. A COMPARISON

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ABSTRACT

The separation of color pigments of chili powder was performed on various narrow bore HPLC columns (octadedecylsilica, silica, and polybutadiene coated alumina) using gradient elution and, the retention characteristics of the columns were compared. The separation capacities of silica and polybutadiene coated alumina were lower than those of other columns. The highest number of fractions were separated of octadecylsilica columns, however, the efficiency of the column depended considerably on the type of C_{18} coating, proving the marked influence of coating process on the separation capacity.

INTRODUCTION

The quantity and composition of color pigments in foods considerably influence the consumer choice and consequently, the marketability of the product. Although spectrophotometric methods, including multiwave length techniques, are

suitable for the determination of the overall quantity of pigments they are unable measure the individual pigment fractions (1,2). As the stability of pigments against oxidation and other storage conditions may be different, the exact knowledge of the pigment composition may help in more accurate prediction of the shelf life of the products. Furthermore, the quantity and quality of pigments may be applied for the authenticity tests of foods.

Because of the marked commercial importance, many liquid chromatographic methods were developed successfully for the separation and quantitative determination of pigments in foods and food products (3). Although the overwhelming majority of methods employed high performance liquid chromatography (HPLC), paper (4) and thin-layer chromatography (TLC) (5), high performance TLC (6), and capillary electrophoresis (7,8) have also been used for the analysis of pigments.

The beneficial effect of carotenoids on human health (9,10) necessitated their determination in various foods and food products using various HPLC methods. Thus, fresh vegetables (11), green leafy vegetables (12), mixed vegetable material (13), cereal products (14), tomato-based food products (15), vegetable (16), tomato (17), and orange juices (18), paprika fruits (19), and oleoresin (20), crude sausage (21), and poultry feed supplement (22) have been investigated.

The majority of HPLC methods applied various reversed-phase supports (23), however, normal phase HPLC has also been applied (24).

The considerable advantages of narrow-bore and microbore HPLC comuns (higher theoretical plate number, lower solvent consumption, shorter analysis time, etc.) resulted in their growing acceptance and application in many field of HPLC analysis. Surprisingly, these columns have not been frequently employed in the analysis of color pigments, only the use of an octadecylsilica (ODS) column of 125 × 3 mm I.D. for the separation of flavonol aglycones and glucosides (25), and the employment of an ODS column of 250 × 2 mm I.D. for the measurement of triphenylmethane dyes in trout muscle (26) was reported.

The objectives of our work were the development of new normal and reversed-phase HPLC methods for the separation and quantitative determination of the color pigments of chili powder using narrow-bore columns, and the evaluation and comparison of the performance of the columns. The number of studies dealing with the comparison of HPLC columns for the separation of color pigments is surprisingly low. It was established that the performance of ODS columns depended considerably on the type of column (27); and the separation of pigments was better on ODS than on silica supports (28).

EXPERIMENTAL

One gram of commercial chili powder (Cholula, Mexico) was shaken for 30 min with 3.0 mL acetone. The suspension was centrifugated and the supernatant

Table 1. Characteristics of HPLC Columns

No. of Column (□m)	Support Type	Particle Size	Dimensions mm	Origin	Carbon Loading
1	ODS	5	150 × 4	Merck	9.7
2	ODS	4	150 × 2	Waters	9.5
3	Silica	4	150 × 2	Waters	–
4	ODS	5	125 × 2	Merck	4.9
5	RP-Alu	5	150 × 2	Merck	4.0

Commercial names: 1 = Hypersil ODS; 2 = Novapack C_{18}; 3 = Silica; 4 = Purospher RP-18; 5 = Aluspher RP-18 (polybutadiene-coated alumina). E. Merck, Darmstadt, Germany; Waters Division of Millipore, Millipore Corporation, Milford, MA.

was separated. This procedure was repeated as the rest of the solid was nearly white. The collected supernatants were evaporated to dryness and dissolved in 10 mL of mobile phase. Each solvent was purchased from Merck (Darmstadt, Germany) and was of HPLC quality.

HPLC separation of pigments was performed with a Waters LC Module I HPLC instrument with a variable injection device and a Waters 746 Data Module integrator (Waters-Millipore Inc., Milford, Massaschuttes, USA). The characteristics of columns and the composition of gradient elutions are compiled in Tables 1 and 2. Columns were not thermostated; each analysis was performed at room temperature (21 ± 1°C). In order to determine the ratio of yellow and red pigments, each measurement was carried out at 340 (yellow pigments) and 440 nm (red pigments).

Each determination was run in triplicate and the relative standard deviation (R.S.D.%) of the retention times and peak areas were calculated (intra-day

Table 2. Gradient Elution Systems. Numbers Refer to HPLC Columns in Table 1

No. of Column	Flow-rate (mL/min)	Eluents		No. of Program
		A	B	
1	0.80	80%Met + 20% ACN	water	I
2	0.17	80%Met + 20% ACN	water	I
3	0.22	hexane	tetrahydrofuran	II
4	0.50	80%Met + 20% ACN	water	I
5	0.17	80%Met + 20% ACN	water	I

Met = methanol; ACN = acetonitrile. Program I = initial composition: 15% A, to 40 % in 10 min, to 80% A in 15 min, 10 min hold, to 90% A in 10 min, 10 min hold, to 97% A in 3 min, final hold; II = initial composition: 0 % B, to 25 % B in 25 min, final hold.

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reproducibility). The inter-day reproducibility was calculated from the three parallel determinations carried out for 4 consecutive working days. In order to find the differences between the stability of columns, the values of standard deviations were compared with the "F" probe.

RESULTS AND DISCUSSION

Large differences were found between the separation efficiency of the various columns. No relationship was found between the efficiency of separation of columns and their theoretical plate numbers determined by model compounds. This finding indicates that, in the case of multicomponent natural mixtures as color pigments, the theoretical plate number is a poor indicator of the separation capacity of the column, and the choice of column cannot be based on its theoretical plate number. The number of yellow and red pigment fractions separated by the columns are listed in Table 3.

Normal phase silica column separated less pigment fractions than octadecyl-coated silica columns did. This finding indicates that color pigments of chili powder show higher differences in their lipophilicity than in their adsoption capacity, therefore, they can be better separated under reversed-phase than normal phase conditions. Interestingly, polybutadiene coated alumina was also not suitable for the separation of pigments, although, it is a reversed-phase column. This result can be tentatively explained by the assumption that the supports partially retain their original adsorption characteristics even after coating with inert hydrophobic ligand.

The secondary polar interactions between the adsorption centers of the stationary phase not covered by the hydrophobic ligand, and the hydrophilic substructures of pigment molecules, may increase or decrease the efficiency of separation.

The chromatograms of pigments obtained on columns 1, 2, and 3 are shown in Figures 1, 2, and 3, respectively.

The chromatographic profiles on columns 1 and 2 are similar, but not identical, the slightly higher number of separated fractions on column 2 may be due

Table 3. Number of Yellow (340 nm) and Red (440 nm) Pigments of Chili Powder Separated by Various Narrow Bore Columns. Numbers refer to HPLC column in Table 1

No. of Column	Yellow Pigments (340 nm)	Red Pigments (440 nm)
1	82	105
2	126	109
3	37	36
4	102	79
5	34	49

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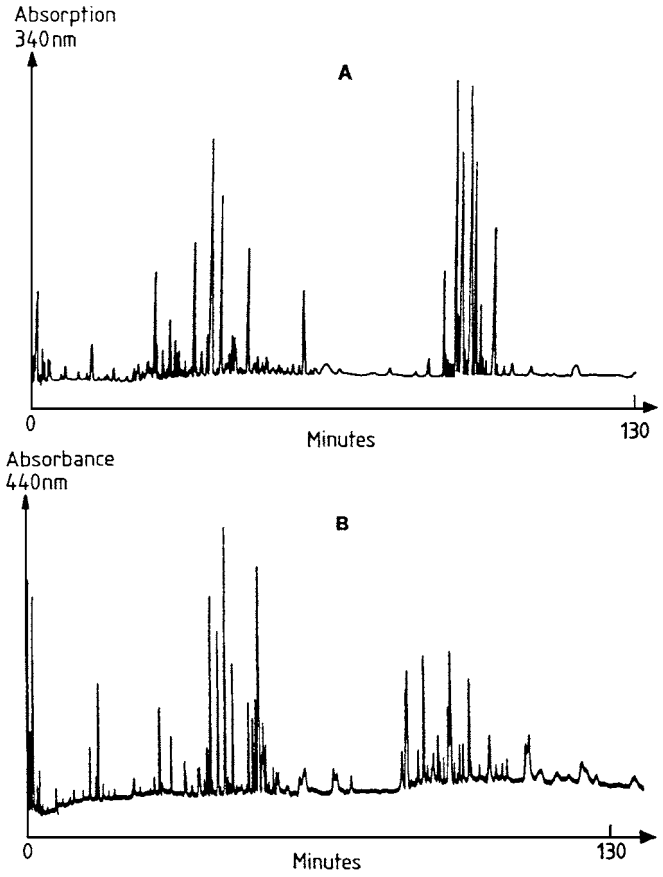

Figure 1. Separation of color pigments of chili powder on a Hypersil ODS column at 340 (A) and 440 nm (B). For conditions see **Experimental.**

to the smaller internal diameter of the column. Pigments form two groups on each chromatogram, a less hydrophobic fraction with lower retention times and a well separated, highly hydrophobic fraction eluting near the end of the chromatogram. This distribution of pigments indicates that they form two chromatographically distinct clusters.

It has to be emphasized that the chromatographic discrimination of fractions does not necessarily mean the similarity of the chemical structures of the pigments belonging to the first or second fraction. The lower retention times on column 3 are probably due to the lower carbon loading of stationary phase. It is possible that using a less steep gradient elution, its separation capacity will be similar to those of columns 1 and 2. It can be established from the comparison of the chromatographic

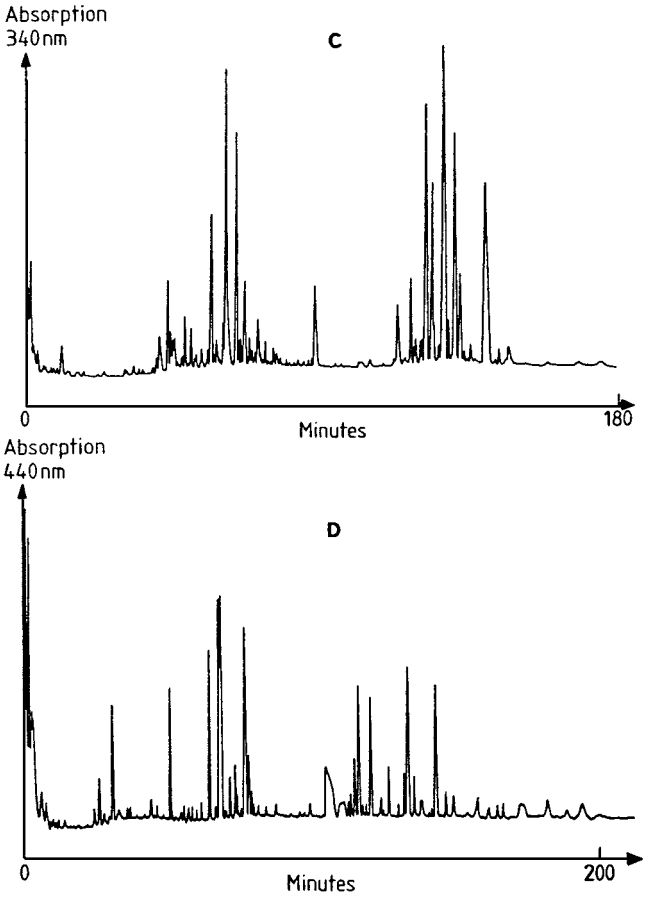

Figure 2. Separation of color pigments of chili powder on a Novapack C_{18} column at 340 (C) and 440 nm (D). For conditions see **Experimental.**

profile of color pigment of chili with that of color pigments of paprika, that the profiles are markedly different and chili powder contains more yellow pigments than paprika does.

No significant differences were found between the intra-day and inter-day reproducibilities of retention times and peak areas proving the similar stability and reproducibility of column. The R.S.D. values for both intra-day and inter-day reproducibilities were 0.6–2.1% for retention times and 3.1–5.7% for peak areas. The relatively high R.S.D. values for peak areas reflects the inadequate separation of some pigment fractions.

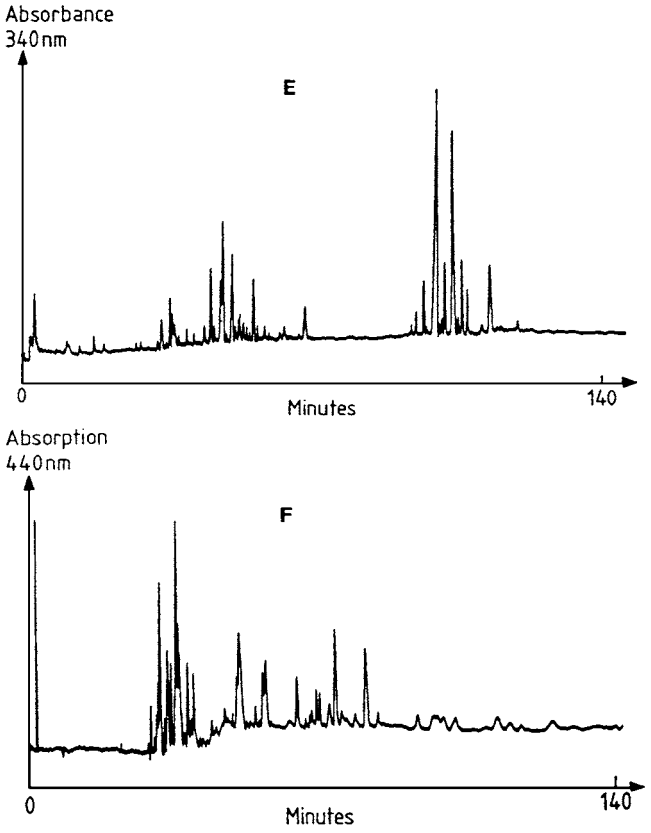

Figure 3. Separation of color pigments of chili powder on a Purospher RP-18 column at 340 (E) and 440 (F) nm. For conditions see **Experimental.**

It can be concluded from the data, that the color pigments of chili powder can be successfully separated in reversed-phase narrow-bore HPLC column using gradient elution. The chromatographic profiles can be used for the evaluation of chili powders and for their authenticity test.

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