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THE ANALYSIS OF ASPIRIN AND RELATED COMPOUNDS BY LIQUID CHROMATOGRAPHY

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SUMMARY

Conditions for the routine separation of the common components of analgesic tablets have been developed. Caffeine, aspirin, 4-hydroxyacetanilide, 4-ethoxyacetanilide and salicylamide have been quantitatively determined in the presence of one another. Twelve different commercial analgesic tablets were analyzed.

INTRODUCTION

The choice of performing an analysis by gas or liquid chromatography points out the complementary nature of these two techniques. If the compounds of interest are thermally stable and volatile, gas chromatography (GC) is the technique of choice; if not, then a liquid chromatographic (LC) analysis should be sought. Analysis of the common components of analgesic tablets is an excellent example of such a choice. Analgesic tablets are usually mixtures of caffeine; aspirin (acetylsalicylic acid); APAP (4-hydroxyacetanilide); phenacetin (4-ethoxyacetanilide); and salicylamide, plus some antihistamine. Of these compounds, only caffeine may be separated by GC without formation of a derivative. The active hydrogens on the carboxyl, amide and phenolic groups inhibit the volatility and stability of the remaining compounds sufficiently to preclude analysis by GC (ref. 1,2).

For these compounds, LC analysis offers the potential of direct analysis with a minimum of sample handling. Since all the compounds contain aromatic chromophores, they are expected to absorb strongly in the ultraviolet, thus facilitating their measurement with an ultraviolet (UV) absorption detector operating at 254 nm.

Recently, a rapid method for the LC analysis of analgesic tablets has been reported[3]. APAP and phenacetin, however, emerged with similar retention times. Since the molar absorptivities of these compounds at 254 nm are different, independent qualitative information would be required for accurate quantitative analysis. Although APAP and phenacetin are seldom encountered in the same tablet, the ability

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to obtain simultaneously qualitative and quantitative information is usually required in screening and analyzing competitive products.

Computerized data processing systems are commonly used in analytical laboratories to prepare analytical reports from gas chromatographs. Such systems are of similar interest in analytical LC. For this reason, the utility and performance of a computerized system were also investigated. Thus, in a study of the analysis of analgesic tablets, we desired to develop conditions for the rapid LC of the expected components of analgesic tablets under conditions suitable for screening applications which require computerized data processing and simultaneous qualitative and quantitative information.

EXPERIMENTAL

Buffer

1.0 *M* Tris pH 9.0. Tris (hydroxymethyl)-amino ethane (121.1 g) was dissolved in 900 ml of distilled water. After adjusting the acidity to pH 9.0 with aqueous hydrochloric acid, the solution was diluted to 1000 ml.

Reference compounds

Samples of pure caffeine, aspirin, APAP, phenacetin and salicylamide were purchased from commercial sources. Individual reference solutions and a mixed standard were prepared by dissolving the desired quantity(ies) in methanol.

Commercial samples

The samples were obtained from local drug stores. The individual components of the commercial samples are listed in Table II. The manufacturers or distributors of the individual tablets are: (A) Bayer aspirin, Sterling Drug, Inc.; (B) Anacin, Whitehall Laboratories; (C) Excedrin, Bristol-Myers Co.; (D) Tylenol, McNeil Laboratories; (E) B.C., B.C. Remedy Co.; (F) Midol, Glenbrook Laboratories; (G) Empirin, Burroughs Wellcome & Co.; (H) Cope, Sterling Drug, Inc.; (I) Bufferin, Bristol-Myers Co.;

TABLE I

QUANTITATIVE STUDIES ON ANALGESIC STANDARD

Injection No.	*Peak area (cm^2)*				
	Compounds				
	Caffeine	*Aspirin*	*APAP*	*Phenacetin*	*Salicylamide*
1	32.72	10.31	30.80	18.00	27.06
2	32.95	10.44	30.92	18.46	28.09
3	32.54	10.18	31.00	17.91	27.19
4	32.33	10.20	30.49	17.82	26.53
5	32.72	9.94	30.49	17.72	27.27
Area average	32.97	10.27	30.74	17.94	27.25
σ	0.23	0.182	0.241	0.289	0.560
% Rel. S.D.	0.70	1.77	0.79	1.62	2.05
Response factor (area/μg)	12.73	2.00	36.11	37.22	13.62

(J) Sentrol, Hudson Products; (K) Excedrin P.M., Bristol-Myers Co.; (L) Safeway aspirin, Safeway, Inc.*.

For analysis, a single tablet was weighed and dissolved in 100 ml of methanol. A 5 to 10 μl aliquot was taken for injection. The amount was varied in order to obtain an absorbence of between 0.20 and 0.64.

Instrumentation

A Varian Aerograph Model LCS-1000 liquid chromatograph was used for the analysis. This instrument is a commercial adaptation of the instrument developed by HORVATH *et al.*[4]. The instrument consists of a column pump, UV absorption detector, columns and oven plus associated controls.

The column was constructed from 300 cm of 1/16 in. O.D. stainless steel tubing with a 1 mm I.D. The tube was packed with lot LFS pellicular anion-exchange resin. A flow rate of 8.6 ml/h generated a back pressure of 925–1000 p.s.i. at 60°. Assuming a void volume of 50%, the linear velocity was 0.61 cm/sec. The LCS-1000 was operated in the single eluant mode. Thus, no regeneration of the column was required between chromatographic runs.

Anion-exchange chromatography was chosen because all the compounds except caffeine exhibit at least one proton which might be ionizable in the mild base. This phenomenon also would be expected to increase a compound's molar absorptivity and, therefore, the sensitivity according to Beer's law.

The pellicular ion-exchange resin is a solid glass sphere about 40 μ in diameter with the anion-exchange resin chemically bonded to the glass. The porous resin shell is about 1 μ thick[5].

To introduce a sample, the eluant flow was stopped, the pressure reduced, and the injection port cap replaced with the syringe guide. The sample was then injected onto the pre-column with a Hamilton syringe. The syringe guide was then replaced with the injection port cap and the sample eluted at a flow rate of 8.6 ml/h. The signal from the chromatograph was displayed on a strip chart recorder. A Varian Data System 200 was used to integrate the peak areas and prepare the report. With the Data System 200, the acquired data were stored until released. This allows the processing of the data by alternate programs, depending on the specific chromatogram. The system was used to perform baseline correction, assign an identity to a peak on the basis of retention time and corresponding response factor, and normalize composition. The computer was also used to calculate the individual response factors.

Response factors

Five consecutive injections of a known standard solution were made. Response factors, (area per μg), for each compound were calculated by dividing the quantity (in μg) into the peak area which was calculated by the Data System. These factors were used to calculate the composition of a known mixture.

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* The identification of the commercial products is solely for the purpose of demonstrating the utility of this method to actual materials of commerce. No conclusions about the relative merits of the individual formulations should be drawn from this work.

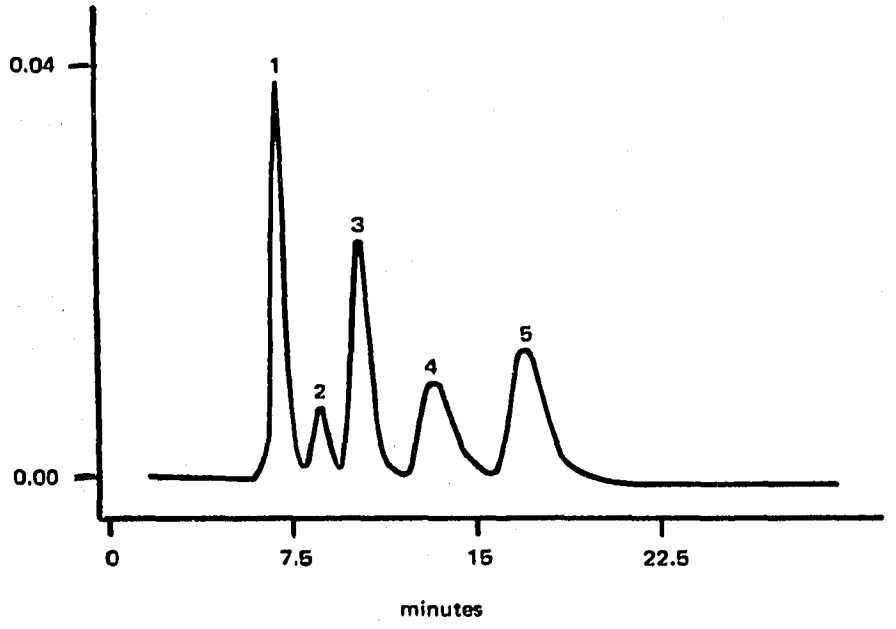

Fig. 1. Liquid chromatography of common components of analgesic tablets. Flow rate, 8.6 ml/h; eluant, Tris (pH 9.0) at 60°. 1 = caffeine; 2 = aspirin, 3 = APAP; 4 = phenacetin; 5 = salicylamide.

RESULTS AND DISCUSSION

The separation of the five common components of analgesic tablets in 20 min is illustrated in Fig. 1. The elution order is caffeine, aspirin, APAP, phenacetin, and salicylamide. The resolution (R) between adjacent peaks is 2.1, 1.3, 1.7, and 1.4. R was calculated using the equation $R = 2d(w_1 + w_2)$ where d is the distance separating the peak maxima and w_1, w_2 the peak widths at the base for each peak. The elution time can be reduced to less than 20 min if one is interested in less than all five components (Fig. 2). This is often the case since most analgesic tablets do not usually contain al five compounds. For example, quality control and formulation studies might require more rapid analyses where the qualitative identification is known independently. Increasing the flow rate to 27 ml/h, caffeine, phenacetin, and salicylamide are separated in less than 10 min (Fig. 2). This required a system pressure of 3000 p.s.i.

Alternately, one of the compounds which is not found in the sample could be added as an internal standard. This would offer the possibility of improved precision in the analysis.

The performance of the method was tested for commercial samples. Twelve commercially available tablets were chromatographed (Fig. 3). These chromatograms show that the method is applicable to the tablets' formulation and no interferences were observed. Each peak is clearly resolved and identifiable.

Other compounds, such as antihistamines or buffering agents are occasionally included in the formulation of analgesic tablets. Little interference was noted from the buffering agents since they do not absorb appreciably at 254 nm. The small peak appearing at 15 min in chromatograms H and K (see Fig. 3) is possibly due to the antihistamine, methapyrilene fumarate. An authentic samples was not available to con-

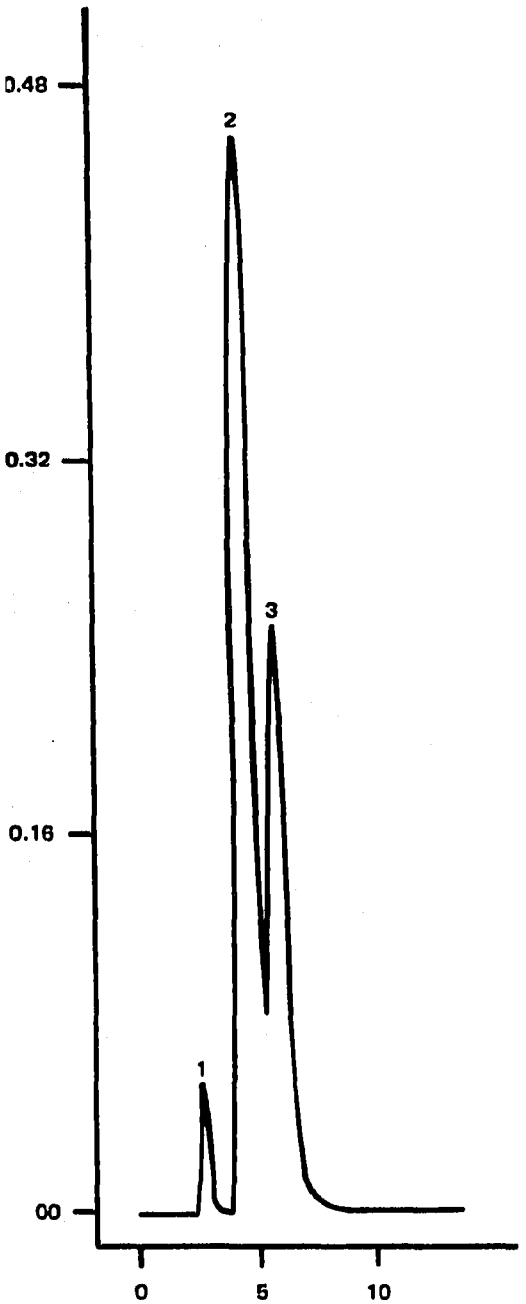

Fig. 2. Rapid liquid chromatography of analgesic tablets. Flow rate, 27 ml/h; pressure, 3000 p.s.i.; temperature, 60°; eluant, Tris (pH 9.0). 1 = caffeine; 2 = phenacetin; 3 = salicylamide.

firm this possibility. Tablet F contained a stimulant, cinnamedrine hydrochloride, which apparently did not elute under these conditions.

Quantitative analysis

Since the output of the UV absorbance detector is linear in absorbance, the area of the individual peaks is proportional to concentration as long as Beer's law is obeyed. Thus, it is possible to use any of the customary GC quantitative techniques, such as peak height, ball and disc, electronic integrator or dedicated computer. The

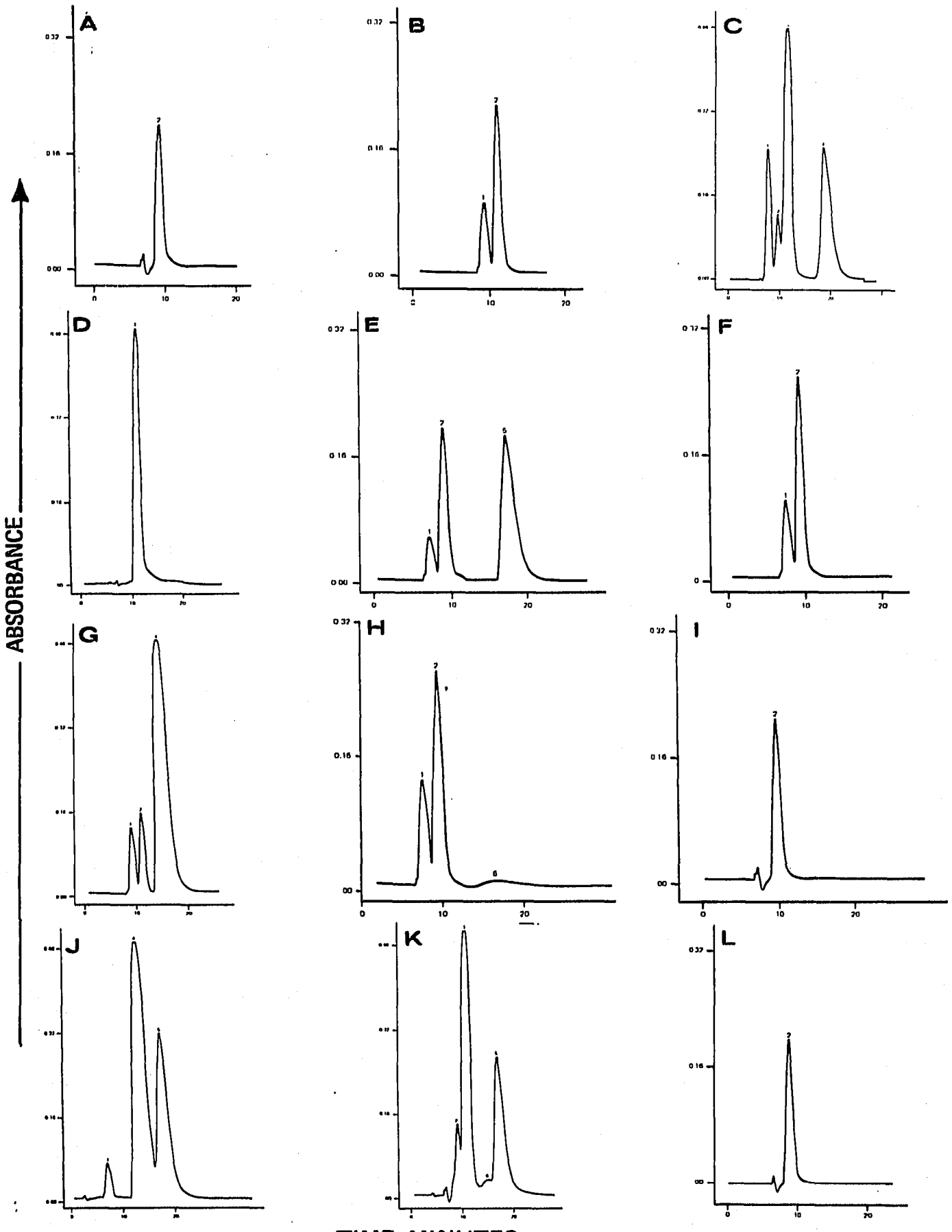

Fig. 3. Chromatography of twelve commercial analgesic tablets. See EXPERIMENTAL section for product identification. 1 = caffeine; 2 = aspirin; 3 = APAP; 4 = phenacetin; 5 = salicylamide. Flow rate, 8.6 ml/h; pressure, 925–1000 p.s.i.

TABLE II

QUANTITY OF FIVE ANALGESICS CONTAINED IN COMMERCIAL ANALGESIC TABLETS

Tablet	*Quantity mg/500 mg tablet*				
	Caffeine	*Aspirin*	*APAP*	*Phenacetin*	*Salicylamide*
A		436			
B	28	450			
C	71	231	81		128
D			112		
E	16	351			88
F[a]	25	428			
G	37	290		150	
H[b]	26	348			
I[c]		337			
J	20			140	206
K[d]		194	89		100
L		500			

[a] Also contains cinnamedrine hydrochloride (stimulant).
[b] Also contains methapyrilene fumarate (antihistamine).
and aluminum hydroxide (gastric antiacid).
[c] Also contains aluminum glycinate and magnesium carbonate (both are gastric antiacids).
[d] Also contains methapyrilene fumarate (antihistamine).

latter affords the maximum in convenience. In this study, a computer was used routinely to compute the peak area and analyze the data, thereby saving an appreciable amount of operation time.

The computer calculated response factors in area per μg from five replicate injections of standard solutions. The results of five consecutive injections (Table I) show relative standard deviations ranging from 0.70% to 2.05% with an average of 1.35% over the five compounds.

The quantity of each component of the analgesic tablets was determined from the peak areas obtained from the chromatograms. These values were multiplied by the appropriate response and dilution factors. For comparison purposes, the composition of each tablet was corrected to a standard tablet weight of 500 mg (Table II). These results were found to correspond well with the contents reported on the label.

TABLE III

REPRODUCIBILITY OF LIQUID CHROMATOGRAPHY OF ANALGESIC TABLETS

[Retention times (sec)]

Compound	*Run No.*					*Mean*	*Dev.*	*%Dev.*
	1	*2*	*3*	*4*	*5*			
Caffeine	432	434	440	435	430	434	3.76	0.87
Aspirin	544	548	555	551	541	548	5.54	1.01
APAP	645	649	659	653	641	649	6.98	1.08
Phenacetin	825	829	843	834	817	830	9.74	1.17
Salicylamide	1045	1051	1066	1053	1038	1051	10.41	0.99
							Average	1.02

The amount of aspirin was usually somewhat higher than expected but the APAP and phenacetin levels were generally lower. Some of the difference between the active ingredients and total weight is probably due to the binding material used in the formulation.

To determine the variance in composition between tablets, the quantity of aspirin in three Bayer tablets was determined. These three values were found to be 436, 458, and 441 mg of aspirin per 500 mg tablet, respectively. The mean for these three values was calculated to be 445 mg with a relative standard deviation of 2.7%.

Qualitative analysis

The qualitative analysis was based solely upon the comparison of retention times of authentic samples with those of the commercial tablets. Of course, this is no guarantee that another compound might have a similar retention time and thus be mistaken for one of the expected compounds. The high precision of the retention times of the standard (Table III), however, minimizes this risk since the probability of making a mistake in identification should decrease as the retention time precision increases.

Data processing

The use of the computerized data processing system, Data System 200, saved a significant amount of time since the only operator time required was for sample preparation, injection and receiving the final report.

Chromatography

The mechanism responsible for the chromatographic separation is probably not purely anion exchange because phenacetin, which lacks functional groups with appropriate pK_a values, is not ionized at pH 9.0, yet is strongly retarded by the column (Fig. 1). Perhaps some interaction between the aromatic rings of the styrene resin and phenacetin is responsible for the separation[6,7]. It is doubtful that adsorption effects between phenacetin and the glass core of the resin bead are responsible because the resin coats the surface of the glass of the pellicular resin. Indeed, microscopic examination of the individual ion-exchange resin beads indicates that the coating is complete and remarkably uniform. Presumably, the bonding of the resin to the bead would also block most of the potential adsorption sites.

CONCLUSION

This paper demonstrates that the five common components of analgesic tablets may be separated rapidly with minimum operator attention to sample preparation. All components are well resolved from each other, facilitating qualitative and quantitative analysis. Computerized data processing has been found to be useful in LC.

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