Automated High-Pressure Liquid Chromatographic Analysis of Aspirin, Phenacetin, and Caffeine

PETER P. ASCIONE ˣ and GEORGE P. CHREKIAN

Abstract □ An automated high-pressure liquid chromatographic (HPLC) method for the separation and determination of aspirin, phenacetin, and caffeine in pharmaceutical dosage forms is described. Separation of these compounds for quantitation is achieved on a controlled pore glass support, utilizing a mixture of acetic acid and chloroform as the mobile phase. The method is specific, accurate, and simple and provides for the quantitation of each chromatogram in a continuous fashion every 7 min. HPLC separation of other analgesics was studied on a spherical siliceous support. The feasibility of determining free salicylic acid in analgesics also was established.

Keyphrases □ Aspirin, phenacetin, and caffeine—automated high-pressure liquid chromatographic analysis □ Phenacetin, aspirin, and caffeine—automated high-pressure liquid chromatographic analysis □ Caffeine, aspirin, and phenacetin—automated high-pressure liquid chromatographic analysis □ High-pressure liquid chromatography—automated analysis, aspirin, phenacetin, and caffeine

The separation and determination of analgesics in pharmaceutical dosage forms have been difficult and time consuming. Column chromatography has been used as an official method for determining aspirin, phenacetin, and caffeine (1, 2), and numerous other methods including solvent extraction techniques for separation of components were reported (3, 4). Other methods for determining the various analgesics involve UV spectrophotometry (5, 6), IR spectrophotometry (7, 8), titration (2), and NMR spectroscopy (9).

The utilization of high-pressure liquid chromatography (HPLC) in pharmaceutical analysis of analgesics was first reported by Henry and Schmidt (10). A recent paper (11) reported conditions for the routine separation of common components of analgesic tablets. The determination of analgesics by HPLC overcomes many shortcomings of the previously reported methods (1–9).

The present work describes the automation of HPLC as applied to the separation and determination of aspirin, phenacetin, and caffeine. It also includes the chromatography of other analgesics and/or combinations amenable to separation and analysis by automated HPLC. By modifying the chromatographic conditions, a rapid quantitative analysis of free salicylic acid can be made.

EXPERIMENTAL

Instrumentation and Reagents—*Apparatus*—The apparatus used in the automated HPLC was acquired from commercial sources and modified as required. The following were used: a solvent delivery pump[1]; a modified sample injection valve[2]; a UV detector equipped with optical path at 254 and 280 nm[3]; a modified liquid sampler II, proportioning pump, and assorted tubing and fittings[4]; a multiple-program timer to control the operation of both the sample injection valve and the liquid sampler[5]; pneumatic air valves, 90 psi capacity[6]; pneumatic piston[7]; a digital integrator[8]; and a recorder equipped with a multirange input and variable chart speed[9].

Columns—Columns are commercially available. They are 600 × 2.1-mm i.d. × 0.6-cm (0.25-in.) o.d. stainless steel tubing with end fitting containing a 2-μm filter bed support with the following packings: Column 1, controlled pore glass CPG-10-240 A, 200–400 mesh[10]; and Column 2, Corasil II[11], consisting of solid glass cores with a porous silica surface. Each column was dry packed with the support by feeding small increments slowly into the empty column while subjecting it to vibration and sharp vertical raps on a hard surface. The addition, vibration, and tapping were continued until the column was completely packed.

Solvents—Spectral grade and/or analytical reagent grade chloroform, methylene chloride, and acetic acid were used.

Mobile Phase—The mobile phase is acetic acid (up to about 10% v/v) in chloroform of such composition that the retention times, when the chromatograph is operated at a flow rate of 1.0 ml/min at ambient temperature, are: aspirin, 135–145 sec; phenacetin, 220–230 sec; and caffeine, 370–390 sec. The recommended mobile phase is 8% acetic acid in chloroform (v/v).

Aspirin, Phenacetin, and Caffeine Working Standard—Use reference standards of known purity. Weigh accurately about 58 mg of aspirin, 43 mg of phenacetin, and 8 mg of caffeine, and dissolve in 100 ml of mobile phase.

Preparation of Sample (Capsules)—Accurately weigh an amount of sample containing the equivalent of the working standard. Transfer to a 100-ml volumetric flask with the aid of 50 ml of mobile phase. Shake for 10 min and dilute to volume with mobile phase. Then filter a portion through fast flow paper.

Table I—Reproducibility of Peak Areas of Aspirin, Phenacetin, and Caffeine Using Automated HPLC

Sample	Peak Area, μv sec		
	Aspirin, × 10^4	Phenacetin, × 10^4	Caffeine, × 10^4
1	71.21	12.43	42.64
2	71.21	12.45	42.77
3	71.19	12.45	42.44
4	71.15	12.44	42.99
5	71.14	12.45	42.70
6	71.00	12.45	42.44
7	71.10	12.46	42.44
8	71.00	12.45	42.30
9	71.04	12.45	42.66
10	71.08	12.46	42.58
Mean	71.11	12.45	42.58
SD	±0.081	±0.0088	±0.163
RSD	0.11%	0.07%	0.38%

[1] Model 711, Laboratory Data Control, Riviera Beach, Fla.
[2] Model HPSV-20, Chromatronix, Berkeley, Calif.
[3] Model 220, Chromatronix, Berkeley, Calif., or Model 1285, Laboratory Data Control, Riviera Beach, Fla.
[4] Technicon Corp., Tarrytown, N.Y.
[5] Model 540, three poles, 2/3-rpm motor, Conrac Corp., Cramer Division, Old Saybrook, Conn.
[6] Schraeder Division of Scovill, Wake Island, N.C.
[7] Air-Mite Division, Chicago, Ill.
[8] Model 3370B, Hewlett-Packard, Avondale, Pa.
[9] Soltec Corp., North Hollywood, Calif.
[10] Electro-Nucleonics, Inc., Fairfield, N.J.
[11] Waters Associates, Framingham, Mass.

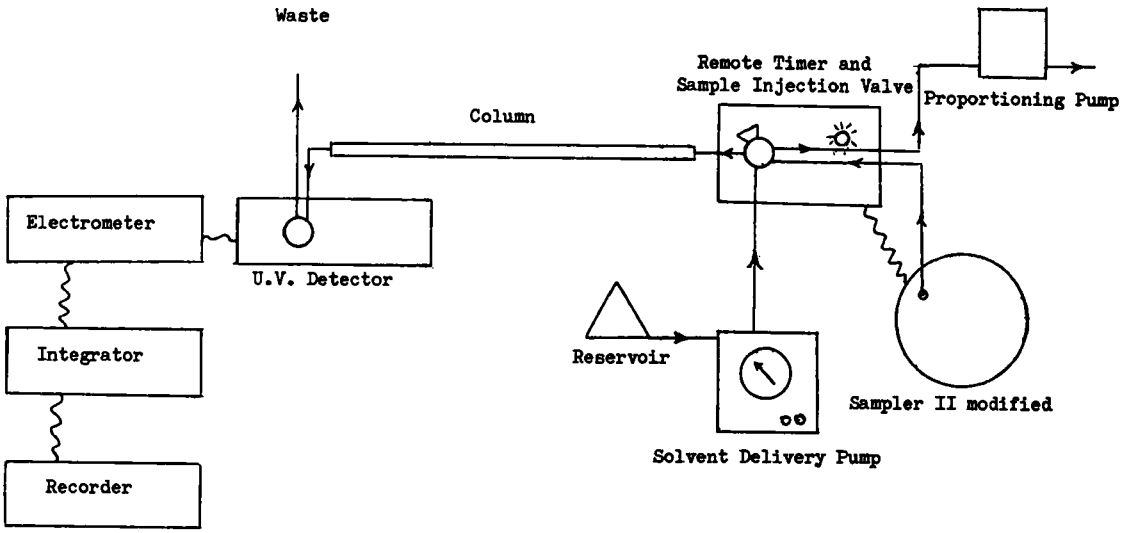


Figure 1—*Schematic diagram of the automated high-speed liquid chromatograph.*

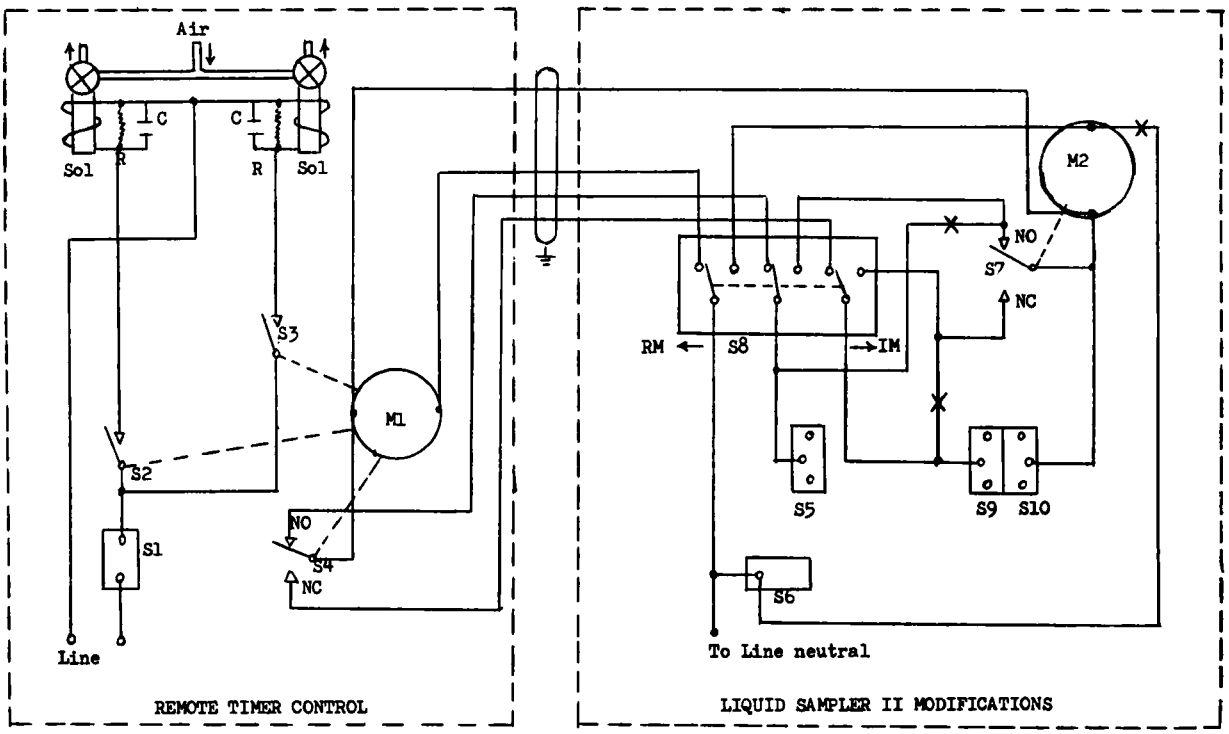
Automated Liquid Chromatographic Procedure—*Chromatographic Conditions*—A steady baseline is established with the following in operation: solvent delivery pump at a flow rate of 1 ml/min, Column 1, UV detector set at 280 nm and a range of 0.32 absorbance unit full scale, integrator settings, slope sensitivity up at 0.1 mv/min, slope down at 0.1 mv/min, peak level at 10 mv, shoulder control at front "on," shoulder at rear 1 mv, baseline reset delay at zero, and recorder with a chart speed of 5 mm/min.

Operating Conditions—Solutions of standards and samples are filled in glass vials, covered with aluminum foil, and placed in the sampler tray. At the same time the proportioning pump and air are turned on to transport the samples from the tray to the injection valve and to activate the pneumatic valves to inject the samples into the liquid chromatograph. Once the chromatographic and operating conditions are established, the control timer and integrator are activated to start the automated analysis. Integration of the chromatographic peak area of samples is compared to areas of standard peaks, and these ratios are used to calculate the analytical results.

RESULTS AND DISCUSSION

This paper describes the automation of HPLC and the chromatographic conditions that optimize the resolution of analgesics in a reasonable time. The automated HPLC system can be represented by the schematic diagram shown in Fig. 1.

The technique consists of a series of integral parts: HPLC sam-



S1-Line Switch
S2,S3-Timer switches SPST
S4-Timer switch SPDT
M1-Remote timer motor
R-22 ohms
C-0.5 microfarads, 600V
S5-Lower probe switch
S6-Alarm switch
S7-Cam switch
S8-Added 3 DT switch
S9-Upper probe switch (outer)
S10-Upper probe switch (inner)
M2-Sampler timer motor
X-Disconnect
RM-Remote Mode
IM-Internal Mode

Figure 2—*Electrical diagram of the remote timer control and liquid sampler II modifications.*

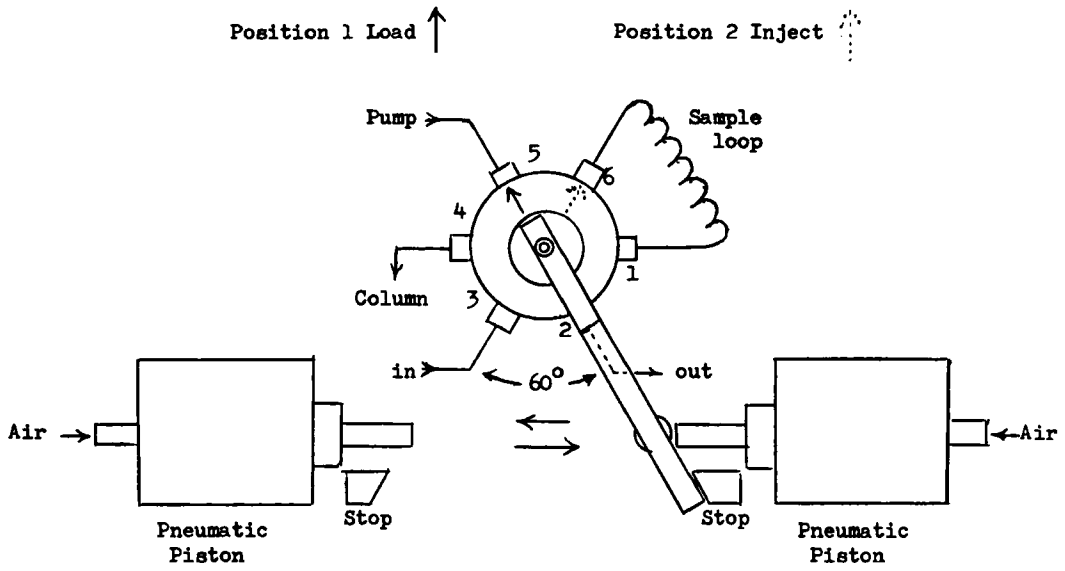


Figure 3—*Automated sample injection valve.*

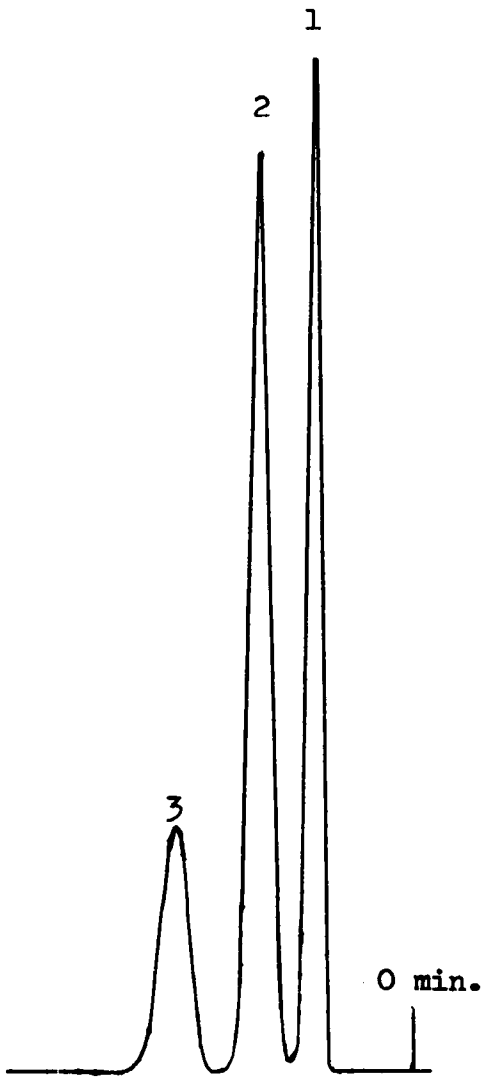


Figure 4—*Separation of aspirin (1), phenacetin (2), and caffeine (3). Chromatographic conditions were: Column 1; mobile phase, acetic acid–chloroform (8:92 v/v); flow rate, 1.0 ml/min; detection, 280 nm; and temperature, ambient.*

pler, sample transport system, automatic injection valve, remote timer control unit, and liquid chromatograph.

HPLC Sampler—A commercial sampler is modified to form a suitable sampler for the HPLC. A three-pole double-throw switch is installed in the sampler to permit a remote timer unit to control the operation of the sampler. This switch permits the use of the liquid sampler in a normal mode and/or by the remote timer control. These modifications are shown in Fig. 2.

Another modification consists of placing a thin sheet of metal on top of the turntable, which is held in place by locating pins. This modification allows glass vials[12] covered with aluminum foil, held in place by a suitable plastic ring, to be placed in the sample tray of the liquid sampler. At the start of the cycle, a sharp probe is lowered into the glass vial, piercing the aluminum foil. The sample is drawn from the vial to the sample injection valve. After injection of the sample, the probe is raised from the vial into a wash reservoir and the turntable is indexed to the next sample. The operation of the probe, turntable unit, and injection valve is controlled by a simple cam timer and microswitches.

Sample Transport System—Transporting samples from the glass vials in the sampler to the sample injection valve is accomplished with a channel peristaltic pump[13] with 0.040 red acidflex tubing. When the sample probe pierces the aluminum foil covering

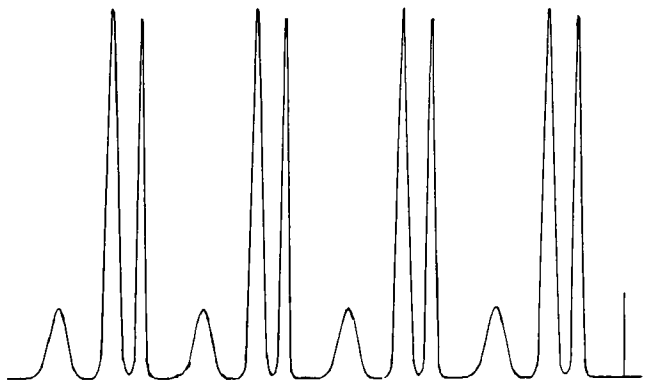


Figure 5—*Reproducibility of automated HPLC separation of aspirin, phenacetin, and caffeine using as a sample the working standard. Chromatographic conditions were: Column 1; mobile phase, acetic acid–chloroform (8:92 v/v); flow rate, 1.0 ml/min; detection, 280 nm; and temperature, ambient.*

[12] Article 60975 (14.5 × 45 mm, 1 dram), Kimble Products, Toledo, Ohio.
[13] Technicon 15.

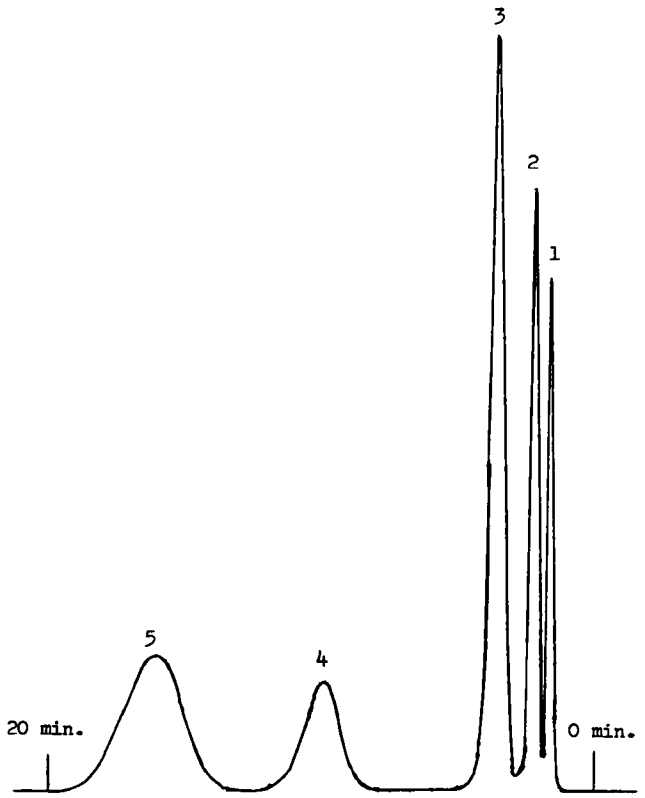


Figure 6—*Separation of five common analgesics. Key: 1, aspirin; 2, salicylamide; 3, phenacetin; 4, caffeine; and 5, acetaminophen. Chromatographic conditions were: Column 2; mobile phase, acetic acid–chloroform–methylene chloride (8:42:50 v/v/v); flow rate, 1.0 ml/min; detection, 254 nm; and temperature, ambient.*

the sample vial, the sample is drawn through the injection valve loop to waste by the peristaltic action of the proportioning pump.

Automated Sample Injection Valve (Fig. 3)—The sample application is accomplished by a rotary switching valve. Operation of the valve from one position to the other is accomplished by pneumatic pistons controlled by the cam timer and microswitch. The commercial sample injection valve is modified to form a suitable injection valve for liquid chromatography by replacing the ceramic rotor with a stainless steel rotor. This valve has an external sample loop, which determines the size of sample applied. Activation is accomplished by pneumatic piston-driven actuators, which position the rotor through the 60° movement. Stops are installed in front of the valve to ensure proper alignment of the flow when the automated valve is rotated fully against each stop (60° apart).

Remote Timer Control—The remote timer associated with the liquid sampler and automated injection valve is used to operate the function of the sampler and to initiate sample injection into the liquid chromatograph (Fig. 2). An electric timer[14] with cams and microswitches is used. Once the precise chromatographic conditions are established, operating procedures for sequential analysis employing the cycle timer are adjusted to control sampling and duration of chromatography.

Chromatography—HPLC is a fast, reliable method for the determination of aspirin, phenacetin, and caffeine. The advantages of this method include fast separation time, sensitive detection, minimum sample preparation time, and precision.

Adsorption, reverse phase, liquid–liquid, and ion-exchange chromatography were evaluated using various supports and mobile phases to obtain the desired separation. Several problems were encountered with these types of chromatography including column bleed, excessive tailing, interferences from sample matrix, and undesirable separation. Analgesics were best chromatographed by the adsorption technique where an acetic acid–chloroform solvent was used with a siliceous stationary phase.

[14] Conrac.

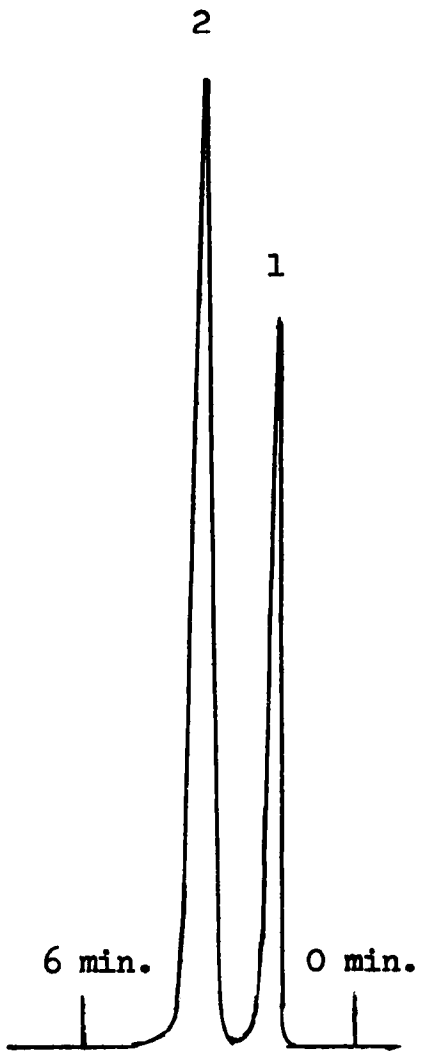


Figure 7—*Separation of salicylic acid (2) and aspirin (1). Chromatographic conditions were: Column 2; mobile phase, acetic acid–methylene chloride (1:99 v/v); flow rate, 1.0 ml/min; detection, 254 nm; and temperature, ambient.*

Figure 4 is a typical chromatogram depicting the separation of aspirin, phenacetin, and caffeine on Column 1. The chromatogram illustrates the range of structures that can be separated with symmetrical peaks using adsorption techniques. Separation is effected by varying the flow rate, changing the ratio of the mixture in the mobile phase, and using various controlled pore glass diameters. Hand-packed columns can be packed homogeneously with a reproducibility in performance of about ±10% within a given lot; however, there are variations in performance from lot to lot of a given packing material. Therefore, minor variations in chromatographic conditions may be necessary to reproduce the work described here.

To determine the precision of the automated procedure, the reproducibility of peak response was studied. A mixture of analgesics was prepared and repeatedly chromatographed. Figure 5 depicts 10 consecutive chromatograms, and a summary of results of integrator areas appears in Table I.

The detector sensitivity range for analgesics appeared to be adequate for most analyses. Linearity of the liquid chromatographic procedure was evaluated by running standard mixtures of various concentrations and plotting these concentrations against their corresponding peak areas. When measured peak areas were plotted, a linear relationship resulted between areas and concentration.

The separation and analysis of other analgesics in combination were applied to automated HPLC. Four common analgesics and caffeine were separated (Fig. 6) using the mobile phase of acetic

acid–chloroform–methylene chloride on Column 2. Aspirin eluted first, followed by salicylamide, phenacetin, caffeine, and acetaminophen. Since most analgesic combinations do not contain all five compounds, slight changes may be made in the mobile phase to reduce the elution time to less than 20 min.

The same chromatographic system can be used for rapid quantitative determination of free salicylic acid. Modifying the mobile phase accomplished the separation of free salicylic acid in analgesics containing aspirin (Fig. 7). This method of separation and detection, with minimum sample preparation, proved to be more rapid than literature methods.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 5, 1974, from the *Pharmaceutical Analytical Development Department, Quality Control Section, Lederle Laboratories, a Division of American Cyanamid Company, Pearl River, NY 10965*

Accepted for publication November 19, 1974.

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Timed-Release Tablets Containing Quinine Sulfate

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Abstract □ The release rates of quinine sulfate from slowly eroding, timed-release tablets prepared with various amounts of a swellable gum, carbomer, and cellulose acetate hydrogen phthalate at different compaction pressures were attained. For the dissolution test of the prepared tablets, the method described in NF XIII was followed. The concentration of the released quinine sulfate was determined spectrophotometrically.

Keyphrases □ Quinine sulfate timed-release tablets—release rates, effect of carbomer and cellulose acetate hydrogen phthalate concentrations □ Carbomer—effect on timed release of quinine sulfate from slowly eroding tablets □ Timed-release quinine sulfate tablets—effect of carbomer concentration on release rate

It has been reported that continuously effective therapeutic drug levels can be achieved over a period of time by incorporating the medicament in a solid, edible, pharmaceutically and medically acceptable vehicle, consisting of a copolymer of glyoxal and partially degraded gelatin, and by compressing the resulting mixture into a tablet. The copolymer was claimed to swell and decompose slowly in the presence of aqueous solutions, liberating the active ingredient gradually (1). A process for the preparation of slowly eroding tablets, involving the compression of a medicinal agent and a hydrophilic swellable gum was described (2). This process was based on the discovery that a gelled zone is formed on the surface when such a tablet comes in contact with aqueous media, which delays entry of water into the interior of the tablet. The incorporation of water-insoluble medicament in a gel, formed from cellulosic gums in organic solvents, was used to produce stable tablet products with delayed release (3).

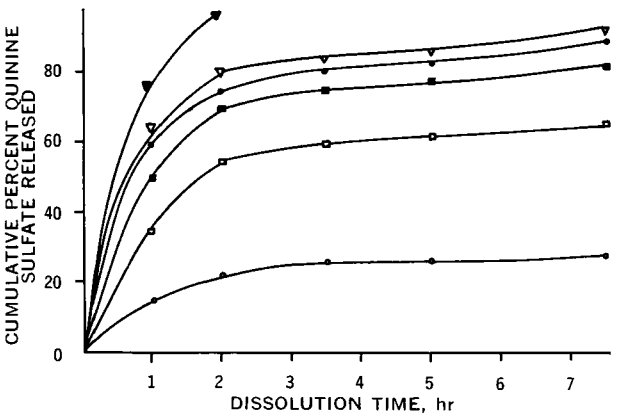

Figure 1—*Drug release rates from timed-release tablets containing decreasing amounts of carbomer. Key:* ○, *Formulation 1.1;* □, *Formulation 1.2;* ■, *Formulation 1.3;* ●, *Formulation 1.4;* ▽, *Formulation 1.5; and* ▼, *Formulation 1.6.*

Compressed tablets were prepared having sustained-release characteristics which contained, in addition to the medicinal agent, a water-insoluble, cross-linked polymer of acrylic acid and a particular, very slightly water-soluble, basic magnesium or calcium compound (4). The sustained mechanism for these polymer-containing tablets was thought to derive from the formation of a protective barrier of gelled material at the tablet surface due to the swelling of the polymer.

The complexity of factors influencing the release of medicament for this category of tablets did not allow the development of an expression to correlate the release rate with physicochemical parameters. It was