tion appears to spread more when the the tablet surface is rough, making the exact determination of the critical surface tension, γc, of a compressed tablet very difficult.

In most cases ($\theta \leq 90°$), the true critical surface tension is greater than that determined experimentally. However, from a film-coating standpoint, the influence of surface effects on film adhesion may well be minimal, with the correlation of solvent–polymer solubility parameters being the major factor in determining film adhesion. Regardless of the tablet surface characteristics, the adhesional bond between tablet and polymer must form at a faster rate than the cohesional forces in the polymer film. When this occurs, a strong adhesional bond is established between the tablet and the film.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 19, 1974, from the *Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907*

Accepted for publication November 5, 1974.

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Laboratory Automation of High-Pressure Liquid Chromatography

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Abstract □ An automated system for high-pressure liquid chromatography was developed. The system is built around commercial modules wherever possible, modified to varying degrees. An automatic sampler, a sample pump, a high-pressure sampling valve, a recorder with an integrator, and a high-pressure liquid chromatograph comprise the commercial instruments. Relays, solenoid valves, and timers control chromatographic events, *i.e.*, duration of sampling and rinse, mobile phase pump refill, sample injection, and chromatographic time. The automated system is dependable over long periods of unattended operation. With the 40-sample capacity of the sample tray and the last sample stop capability, the automated system produces, for example, 40 20-min chromatograms in approximately 13 hr of unattended operation. Data demonstrate the reliability and utility of the system.

Keyphrases □ High-pressure liquid chromatography—automated equipment developed and discussed □ Automated analysis—high-pressure liquid chromatographic equipment developed and discussed

High-pressure liquid chromatography (HPLC), with its widespread use over the last 5 years, has undergone dramatic growth from the standpoint of methodology and instrumentation. Michaelis *et al.* (1) made it apparent that HPLC is of major importance in quantitative analysis and identification of various pharmaceutical agents. An HPLC equipment review (2) covers a large number of domestic and foreign manufacturers.

The application of HPLC for stability-indicating assays because of specificity, sensitivity, and relative ease of sample preparation is clearly indicated, particularly for labile compounds. Specific applications of HPLC were reported for various pharmaceutical products containing various active compounds (3) and for steroid formulations (4–7). The increased use of HPLC for stability-indicating assays of steroid formulations, for example, has produced the need for an automated HPLC system. Specific applications of the automation of particular aspects of HPLC have been reported. Among these are the use of short columns and low pressures (8) and the determination of the antibiotic tetracycline (9). More recent work (10) covers the use of a dedicated small computer to study the precision in HPLC.

Although automated HPLC systems are obtainable from commercial manufacturers[1,2], they are not readily available. A symposium presentation (11) covered an automated HPLC system developed at Lederle Laboratories. To increase the output of HPLC assays, an automated HPLC system (patent applied for) was developed from commercially available instruments, relays, solenoid valves, and timers and is the subject of this report.

EXPERIMENTAL

The commercial instruments comprising a portion of the automated HPLC system, modified to varying degrees, follow.

Instruments and Modifications—A liquid chromatograph[3]

[1] DuPont, Wilmington, Del.
[2] Altex, Berkley, Calif.
[3] Model 820, DuPont.

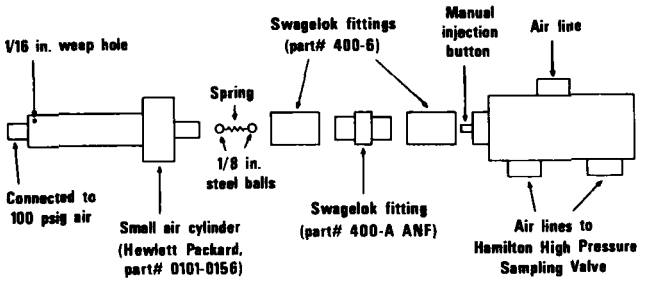

Figure 1—*Schematic drawing showing the modification of the high-pressure sampling valve to make it operate automatically.*

with 316 stainless steel columns, 100 cm long × 2.1 mm i.d., and permanently bonded, reverse-phase column packings[4,5] were used. The chromatograph was equipped with a 254-nm UV detector set at a sensitivity of 0.04 absorbance unit full scale.

A 1-mv recorder[6] was equipped with an integrator[7]. Chart speeds were 0.25 and 0.51 cm/min.

The high-pressure sampling valve[8] provides plug injection of sample at liquid pressures up to 5000 psig. It is mounted in the column oven and is actuated by 30–100 psig of air, supplied from the pneumatic section of the mobile phase pumping system of the chromatograph. A 5.0-μl sample valve was used. The manual injection switch of the high-pressure sampling valve was modified to operate automatically from the 30–100-psig air *via* an air cylinder[9] and a solenoid valve[10]. Figure 1 gives a schematic drawing of the details.

The holdup volume of the sample pump[11] was reduced with Teflon inserts in the entrance and exit chambers. The inserts have openings approximately 0.13 cm (0.050 in.) in diameter for fluid transport. The power plug was changed to a pin type, dedicating it to the automated system.

The standard timing cams were eliminated in the automatic sampler[12]. Sampling time is controlled by an automatic timer[13] located in a separate unit. A metal disk was constructed and placed under the sample tray to act as a support for 45 × 14-mm o.d. glass vials holding approximately 3 ml. A glass cover plate replaced the plastic one provided by the manufacturer. Retaining clips were added to the glass plate and to the sample tray to orient and anchor the cover plate.

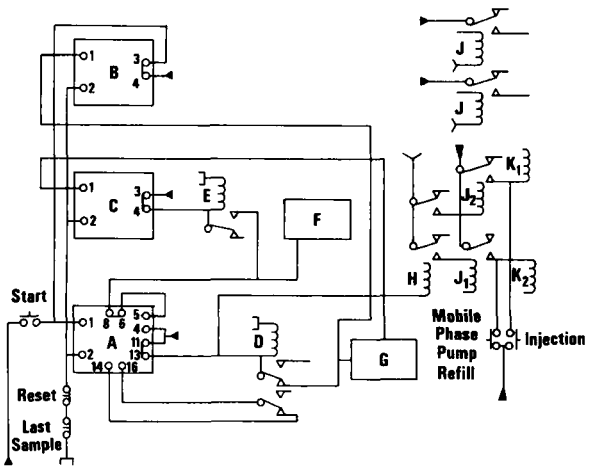

Figure 2—*Schematic drawing showing relative position and wiring of modules comprising the automated HPLC system. Key: ▲, 115 v ac; △, 24 v ac; >, 24 v dc; and], neutral. See text for details.*

[4] DuPont ODS.
[5] Waters C-18.
[6] Electronik 19, Honeywell, Philadelphia, Pa.
[7] Disc Instruments, Santa Ana, Calif.
[8] Hamilton, supplied by DuPont.
[9] Hewlett-Packard, Palo Alto, Calif.
[10] Automatic Switch Co., Florham Park, N.J.
[11] miniPump, Milton Roy, St. Petersburg, Calif.
[12] Sampler II, Technicon, Tarrytown, N.Y.
[13] Model 335, Automatic Timing Controls Co., King of Prussia, Pa.

Table I—Replicate Automated HPLC Injections of 5 μl of a Solution Containing 0.075 mg/ml of Fluorometholone and 0.05 mg/ml of Norethindrone as the Internal Standard

Injection	Peak Height Ratio	Injection	Peak Height Ratio
1	1.3571	18	1.3621
2	1.3571	19	1.3576
3	1.3571	20	1.3702
4	1.3571	21	1.3576
5	1.3571	22	1.3676
6	1.3620	23	1.3624
7	1.3701	24	1.3699
8	1.3527	25	1.3424
9	1.3592	26	1.3564
10	1.3460	27	1.3691
11	1.3484	28	1.3704
12	1.3675	29	1.3684
13	1.3519	30	1.3677
14	1.3599	31	1.3579
15	1.3460	32	1.3484
16	1.3482	33	1.3586
17	1.3519	34	1.3542
		Average =	1.3583
		RSD =	0.61%

Approximately 0.10-cm (0.040-in.) i.d. polyethylene tubing connects the automatic sampler and the sample pump. Stainless steel tubing, 0.03 cm (0.010 in.) i.d., connects this pump to the sampling valve. The same dimension tubing connects the high-pressure sampling valve to the column.

Wiring and Functions of Electronic Components—To control and to coordinate the sequence of events necessary for the flexible operation of the automated HPLC sampling system, appropriate timers, relays, and solenoid valves were installed. Timers were contained in a separate control unit; relays and solenoids were mounted in the chromatograph.

As shown in Fig. 2, three separate timers[13] establish sample, rinse, and chromatographic times. The pump timer (A) is wired for momentary start and resets by the time delay relay (D) contact opening (adjustable 3–30 sec, normally set to 4 sec). The chromatographic timer (B) is wired for momentary start and automatic reset. Depression of the start switch located in the control unit causes the sample pump timer (A) to start timing. This allows the sample pump (F) to run for the preset time.

At the end of the sampling time, No. 8 contacts open, stopping the sample pump; No. 13 contacts close and apply power to the time relay (D), the automatic sampler (G), and the standard 115 v ac relay (H). The sampler (G) starts, and the sample probe is allowed to cycle to the wash position. The time delay relay (D) then times out to keep the sample probe from coming out of the rinse solution. When in the rinse position, the sample pump (F) is re-

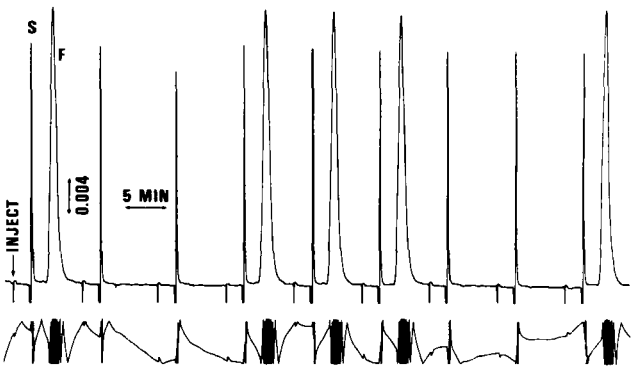

Figure 3—*Eight-minute chromatography from the automated HPLC sampling system showing the absence of carryover from fluorometholone, 0.15 mg/ml (F), or 75% 3A alcohol (S). The sequence of injections, from left to right, was F, S, S, F, F, F, S, S, and F. Chromatographic conditions were: ODS column packing, 6% aqueous acetonitrile with 0.0075 M borate buffer at pH 11.0 as the mobile phase, 500 psig column pressure (0.67 ml/min), and column temperature at 35°.*

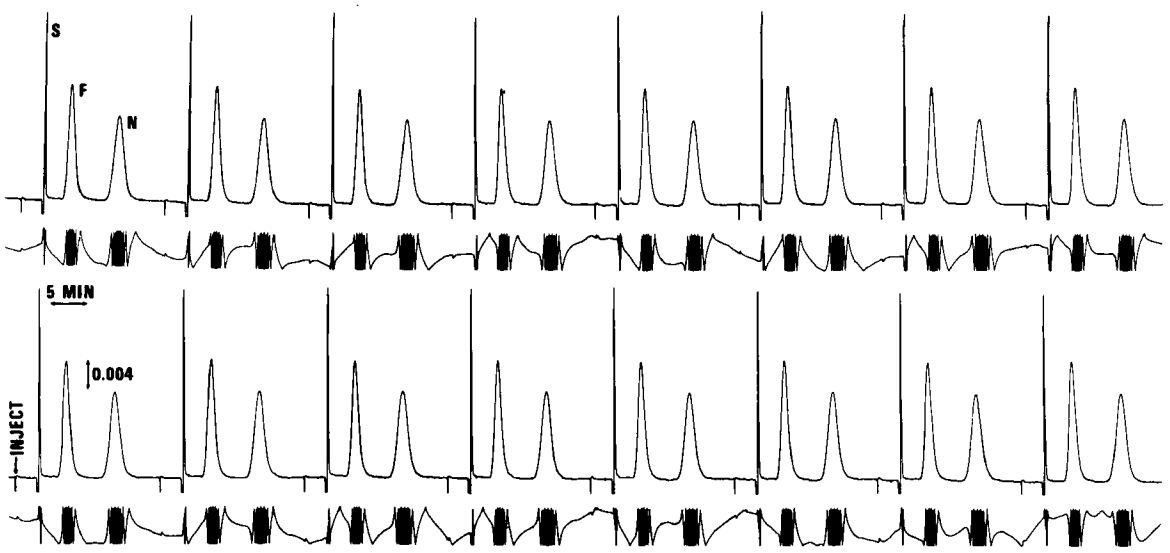

Figure 4—*Twenty-minute chromatograms of automatically sampled fluorometholone, 0.075 mg/ml (F), and internal standard norethindrone, 0.050 mg/ml (N), in 75% 3A alcohol (S). Chromatographic conditions were: ODS column packing, 6% aqueous acetonitrile with 0.0075 M borate buffer at pH 11.0 as the mobile phase, 300 psig column pressure (0.4 ml/min), and column temperature at 35°.*

started *via* a standard 115 v ac relay (E), mounted in the sampler and energized by the rinse timer (C). The timer (C), started by the sampler, operates for the programmed rinse time. After timing out, the sampler probe moves to the sampling position and rests until the next sampling and injection cycle.

The 115 v ac relay (H) closing allows the 24 v dc supply to pull in the mobile phase pump refill relay (J_1) and the sample injection relay (J_2) which, in turn, supplies 24 v ac current to two solenoid valves[10] (K_1 and K_2). K_2 supplies air to refill the mobile phase pump, and K_1 supplies air to the air cylinder, which pulses the injector valve and causes the sample from the flowing stream to be injected. The spring, steel balls, and weep hole ensure return of the plunger after injection to the normally off position.

The two solenoid valves can also be activated *via* front panel push button switches in the manner provided by the chromatograph manufacturer. The time delay relay (D) times out, resets sample pump timer (A), which, in turn, removes 115 v ac from contact 13, removing power from the solenoid valve *via* the relay (H). The chromatograph timer (B), on timing out, restarts the sample pump timer (A) *via* contact 3. The chromatograph time can be stopped at any desired time by manually pushing a reset button or by an automatic stop activated by the last sample in the sample tray. The last sample stop also removes power from the recorder and the mobile phase pump. The UV detector remains on to ensure its stability.

Each of the four functions—control of sample pump, control of automatic sampler, mobile phase pump refill, and sample injection—has a separate relay (J), which can be activated by a 24 v dc signal. This system was used so that the automated HPLC sampling system can be computer operated, independent of the three timers now used. The three timers were wired in the following manner: pump timer, wired for momentary start and separate reset switch; rinse timer, wired for sustained start; and chromatograph timer, wired for momentary start and automatic reset at end of programmed time.

Procedure—The sample probe is placed in a vial of liquid (preferably sample solvent), and the sample pump is primed. The sampling rate is selected from the vernier of the sample pump; sampling and rinse times are set on their appropriate timers. The length of chromatographic time is set, with the exact time determined from a test chromatogram. This time is calculated as the elapsed time from one sample injection to the next sample injection. Sampling time occurs for a preset period prior to injection. Sampling, therefore, can be programmed to begin during the elution of the last peak, depending on what effect, if any, the initiation of sampling signal has on the chromatogram.

The glass sample vials or plastic sample cups can be closed with aluminum foil, if necessary, to prevent solvent evaporation prior to placement in the sample tray. For many applications, however, the glass cover plate is satisfactory, particularly if internal standards are used.

Table II—Replicate Automated HPLC Injection of 5 μl of a 0.25-mg/ml Solution of 9α-Fluoroprednisolone Acetate

Injection	Peak Height[a]	Injection	Peak Height[a]
1	57.3	11	58.0
2	56.9	12	58.7
3	57.7	13	58.1
4	57.5	14	58.3
5	57.7	15	58.0
6	57.9	16	58.0
7	57.5	17	58.9
8	56.4	18	58.5
9	57.7	19	57.4
10	58.1	20	58.0
		Average =	57.8
		RSD =	1.0%

[a]Percent of full chart.

RESULTS AND DISCUSSION

Selections of the sampling rate by vernier adjustment of the sample pump and sampling time with a timer control the sample

Table III—Automated HPLC Analysis of 9α-Fluoroprednisolone Acetate in a Commercial Veterinary Product with and without an Internal Standard

	9α-Fluoroprednisolone Acetate, mg/ml	
Sample	With Internal Standard	Without Internal Standard
1	1.91	2.00
2	2.06	2.00
3	2.01	1.99
4	2.03	1.97
5	2.00	2.00
6	1.98	1.94
7	2.00	1.95
8	2.00	1.99
9	2.02	2.03
10	2.02	2.03
11	2.04	2.01
12	2.01	2.01
Average =	2.01	1.99
RSD =	1.84%	1.41%

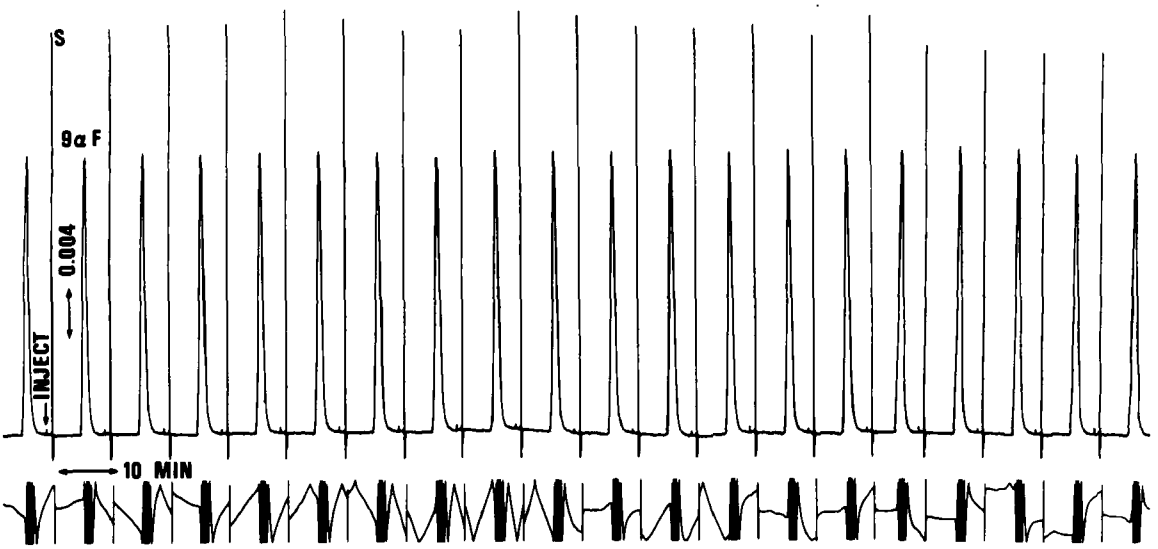

Figure 5—*Ten-minute chromatograms of automatically sampled 9α-fluoroprednisolone acetate, 0.25 mg/ml (9αF), in 75% 3A alcohol (S). Chromatographic conditions were: C-18 column packing, 24% aqueous acetonitrile mobile phase at 1000 psig (1.5 ml/min), and column at ambient temperature.*

volume desired to flush the automated injection system. The volume of sample available for injection was usually 10 ml or more; therefore, volume was no problem. Approximately 3 ml of sample and 3 ml of rinse were used routinely. Settings of 50 on the sample pump vernier and of 2 min on sampling and rinse timers gave these volumes. Figure 3 gives chromatograms showing the absence of carryover when going from a steroid sample, producing a peak approximately 77% of full scale, to 75% 3A alcohol solvent. For these chromatograms, an ODS column at 35° was used with a 6% aqueous acetonitrile mobile phase containing 0.0075 *M* borate buffer at pH 11.0. The flow rate was 0.67 ml/min, and pressure was 500 psig.

These conditions are similar to those used for the assay of fluorometholone in a topical steroid cream[14]. Either 5 μl of fluorometholone at 0.15 mg/ml in 75% 3A alcohol or 75% 3A alcohol was automatically injected. Chromatographic time was set for 8 min. Under the same chromatographic conditions, except for a pressure of 300 psig (0.4 ml/min), fluorometholone (0.075 mg/ml) and norethindrone internal standard (0.05 mg/ml) were automatically injected over approximately 11.5 hr, yielding 34 separate chromatograms, each of 20-min duration. The peak height ratios with a relative standard deviation of 0.61% are shown in Table I. A number of the chromatograms are shown in Fig. 4.

Additional data using the automated HPLC sampling system were obtained using a C-18 column with a mobile phase of 24% aqueous acetonitrile. Column pressure was 1000 psig with a flow rate of 1.5 ml/min at ambient temperature. The compound chromatographed was 9α-fluoroprednisolone acetate in a veterinary product[15] at approximately 0.24 mg/ml in 75% 3A alcohol. The relative standard deviation for 20 separate sample injections was 1.0% (Table II). A reproduction of 20 chromatograms, each of 10 min duration, is given in Fig. 5.

HPLC assays of 9α-fluoroprednisolone acetate, prepared for assay with and without the internal standard, were made using the automated HPLC sampling system. Twelve separate samples were prepared for assay using 2.0 ml of the steroid product and 15.0 ml of the internal standard solution (norethindrone at 0.20 mg/ml in 75% 3A alcohol). These samples were assayed against a 9α-fluoroprednisolone acetate standard (0.25 mg/ml) dissolved in the internal standard solution. Assay samples without the internal standard were prepared by adding 2.0 ml of the steroid formulation to 15.0 ml of 75% 3A alcohol. These samples were assayed against 9α-fluoroprednisolone acetate standard with a placebo vehicle. The standard solution was prepared by adding 2.0 ml of placebo vehicle to 15.0 ml of a 0.25-mg/ml 9α-fluoroprednisolone acetate solution in 75% 3A alcohol. The placebo vehicle was added to the standard solution to compensate for the volume change of the sample preparation. Table III shows that nearly identical results were obtained, with the steroid content of the 12 samples near the labeled amount (2.0 mg/ml).

The data show that the automated HPLC system described is capable of supplying uniform data over a long, unattended period. The data also suggest that the application of automated HPLC assays without the use of internal standards is possible, thus reducing analysis time as well as development time. Peak areas were also available for the data of this report; however, peak heights gave better precision and, therefore, were used in the calculations.

[14] Oxylone, topical cream, 0.025%, The Upjohn Co., Kalamazoo, Mich.

[15] Predef 2X, 2 mg/ml sterile aqueous suspension, The Upjohn Co., Kalamazoo, Mich.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 22, 1974, from *Analytical Research & Development and †Instrumentation, The Upjohn Company, Kalamazoo, MI 49001

Accepted for publication February 5, 1975.

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