Journal of Chromatography, 257 (1983) 337–344
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,492

AUTOMATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMN SWITCHING TECHNIQUE FOR THE ON-LINE CLEAN-UP AND ANALYSIS OF DRUGS IN TOPICAL CREAM FORMULATIONS

D. L. CONLEY and E. J. BENJAMIN*
Institute of Pharmaceutical Sciences Syntex Research, Stanford Industrial Park, Palo Alto, CA 94304 (U.S.A.)
(First received May 24th, 1982; revised manuscript received November 2nd, 1982)

SUMMARY

The automation of a high-performance liquid chromatographic column switching technique for the on-line clean-up and analysis of drug substances in topical cream formulations is described. A short reversed-phase precolumn used for primary sample clean-up was coupled to a reversed-phase analytical column. This allowed cream samples dissolved in methanol–tetrahydrofuran to be injected directly into the analysis system without prior laborious and time-consuming clean-up procedures. This application was successfully extended to the analysis of sulconazole nitrate, and a triple corticoid cream. The effects of precolumns on some chromatographic parameters were studied.

INTRODUCTION

The use of column switching for the clean-up and analysis of multicomponent samples has been reviewed by Apffel *et al.*[1] and Freeman[2]. Various column switching systems involving liquid chromatography (LC) as the clean-up system followed by liquid chromatography as the analysis system (LC–LC systems) have been reported[3–9] for the clean-up or enrichment of aqueous solutions. The first application of a manual on-line column switching system for the clean-up and analysis of a multicomponent topical cream formulation was described by Benjamin and Conley[10]. In this study, the elution of cream excipients from a 3 cm × 4.6 mm I.D. reversed-phase precolumn placed in the sample loop (loop column) was examined as a function of mobile phase composition. It was shown that under the chromatographic conditions of the reversed-phase high-performance liquid chromatographic (HPLC) system used to analyze a given cream, the non-polar excipients were strongly retained on the loop column relative to the drug substances and thus provided an excellent clean-up of the cream samples. This paper discusses the principles and instrumentation of the first applications of an automated column switching technique for the clean-up and analysis of drug substances in topical cream formulations.

0021-9673/83/0000–0000/$03.00

EXPERIMENTAL

Apparatus

The modular HPLC system consisted of a dual piston pump (M6000A, Waters Assoc.), an autoinjector (WISP, Waters Assoc.), a six-port injector valve (Model 7010, Rheodyne, U.S.A.) with a pneumatic actuator (Model 7001, Rheodyne), a solenoid and solenoid interface (Autochrom, U.S.A.), a microprocessor controller (MicroMaster WP-6000, Minarik Electric, U.S.A.), a low-pressure peristaltic pump (Series E, Eldex Labs., U.S.A.), a variable-wavelength detector (LC-75, Perkin-Elmer, U.S.A.) and a recorder/integrator (Data Module 740, Waters Assoc.).

The MPLC™ loop column cartridges (3 cm × 4.6 mm I.D.) and the cartridge holder were obtained from Brownlee Labs, U.S.A. Two prepacked analytical columns were used in this study: a Partisil-10-ODS-3, 10 μm, 25 cm × 4.6 mm I.D. (Whatman, U.S.A.) and an Ultrasphere C_{18}, 5 μm, 25 cm × 4.6 mm I.D. (Beckman, U.S.A.). A guard column, 7.0 cm × 2.1 mm I.D., was dry-packed with Co:Pell ODS (Whatman) and coupled to the analytical column. The automated system was set up as shown in Fig. 1.

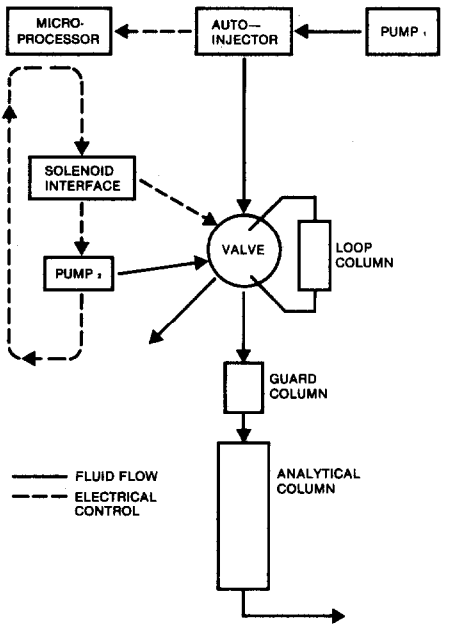

Fig. 1. Schematic diagram of the automated system.

Procedures

Typically, cream samples were dissolved in tetrahydrofuran (THF) and diluted with methanol to obtain a concentration of active compounds which would allow the injection of less than 25 μl of the solution. Larger injection volumes produced shoulders and doublets due to the polarity difference between the injection solution and the mobile phase[11]. When necessary, sample solutions were clarified by centrifugation

TABLE I

SAMPLE DILUTIONS AND COLUMNS USED FOR THE ANALYSIS OF VARIOUS TOPICAL CREAM FORMULATIONS

Cream	*Sample weight*	*Volume of methanol–THF*	*% THF*	*Loop column*	*Analytical column*
Sulconazole nitrate 1%	0.5 g	100 ml	5	Partisil-10-ODS-3, 10 μm	Partisil-10-ODS-3, 10 μm
Triple corticoid Fluocinonide 0.00925% Procinonide 0.00365% Ciprocinonide 0.0021%	5.0 g	25 ml	10	Spheri-5 ODS, 5 μm	Ultrasphere C_{18}, 5 μm

prior to injection. The concentration of THF was 10% or less in the sample solution injected. The sample dilutions for various cream products are listed in Table I.

A schematic diagram of the automated analytical system is shown in Fig. 1. With the six-port injection valve in the "inject" position (loop column on-line), the dissolved sample is introduced into the system by an autoinjector and carried in mobile phase to the loop column. At the time of injection, the autoinjector signals the microprocessor to begin a previously programmed sequence of timed events (Table II). The active compounds elute from the loop column with the mobile phase (pump 1) while the non-polar excipients are retained. The microprocessor then signals the

TABLE II

AUTOMATION SEQUENCES FOR THE ANALYSIS OF VARIOUS CREAMS

Time (min)		*Equipment status*		*Event*
Sulconazole nitrate cream	*Tripole corticoid cream*	*Loop column*	*Pump 2*	
0	0	On-line	Off	Autoinjector injects sample.
0.01–7.00	0.01–7.00	On-line	Off	Active compounds elute from loop column, excipients retain.
7.01	7.01	Off-line	Off	Loop column is switched off-line.
7.50	7.50	Off-line	On	Loop column wash pump (pump 2) is turned on (1.0 ml/min).
7.51–10.49	7.51–12.49	Off-line	On	Excipients are backflushed to waste.
10.50	12.50	Off-line	Off	Pump 2 is turned off;
	22.00*	Off-line	Off	wavelength change to 236 nm for quantitation of last two peaks
	34.50*	Off-line	Off	Return to 260 nm when last two peaks have eluted.
17.00	35.00	On-line	Off	Loop column is switched on-line after all peaks have eluted from the analytical column.
17.00–22.00	35.00–40.00	On-line	Off	Loop column equilibrates in mobile phase.
22.01	40.01	On-line	Off	Sequence returns to RESET.

* For triple corticoid cream only.

TABLE III

EFFECTS OF THE LOOP COLUMN ON CHROMATOGRAPHIC PARAMETERS

N = Theoretical plate count; As = assymetry factor; R = resolution.

Analytical column packing	*Loop column packing*	*Mobile phase*	*Active compound*	*Chromatographic parameters ± standard deviation*				
				Active compound		*Internal standard*		
				N	*As*	*N*	*As*	*R*
Partisil-10-ODS-3, 10 μm	*	Acetonitrile–water–acetic acid (48:51:1) with 0.01 *M* $KClO_4$	Sulconazole nitrate	3334	1.18	2988	1.18	4.2
Partisil-10-ODS-3, 10 μm	Partisil-10-ODS, 10 μm	Acetonitrile–water–acetic acid (48:51:1) with 0.01 *M* $KClO_4$	Sulconazole nitrate	3306	1.44	3196	1.28	4.30
Ultrasphere-ODS, 5 μm	*	THF–acetonitrile–water (4:43:53)	Fluocinonide	11736	1.08	7901	1.11	
			Procinonide	14287	1.09			2.17***
			Ciprocinonide	14722	1.25			
			Fluocinolone acetonide**	4323	1.38			
Ultrasphere-ODS, 5 μm	Spheri-5 ODS, 5 μm	THF–acetonitrile–water (4:43:53)	Fluocinonide	8145	1.43	6016	1.40	
			Procinonide	9724	1.23			1.75***
			Ciprocinonide	9494	1.50			
			Flucinolone acetonide**	3889	1.80			

* Loop column off-line.

** Major degradation product.

*** Resolution between procinonide and ciprocinonide.

activation of the six-port valve placing it in the "load" position (loop column off-line) trapping the excipients. A 30-sec pause is entered in the program at this point as a precaution against loop column bed disturbance due to an abrupt reversal in flow direction. After 30 sec, the microprocessor activates the low-pressure pump (pump 2) which backflushes the loop column with methanol–THF (75:25) at 1.0 ml/min for at least 3 min. The low-pressure pump is then turned off. When all of the active compounds have eluted from the analytical column, the loop column is returned to the on-line position and allowed to equilibrate with mobile phase prior to the next injection.

RESULTS AND DISCUSSION

Analysis of sulconazole nitrate cream

The quantitation of sulconazole nitrate and similar hydrophobic amine salts in biological samples[12,13] and a cream formulation[14] has been accomplished using multi-step sample pretreatment prior to HPLC analysis. A manually controlled system for the simultaneous clean-up and analysis of hydrocortisone and sulconazole nitrate in cream was described previously[10]. In this method, a LiChrosorb RP-18, 10-μm loop column was successfully used in the clean-up of a cream containing the hydrophobic amine. Subsequent lots of this packing material would not allow the elution of the hydrophobic amine possibly due to a strong interaction with residual silanol sites in the packing. The fully derivatized Partisil-10-ODS-3, 10-μm, loop column yielded superior chromatography.

Fig. 2 is a typical chromatogram of sulconazole nitrate and the internal standard (fluocinonide) using the automated method. The use of the loop column had no

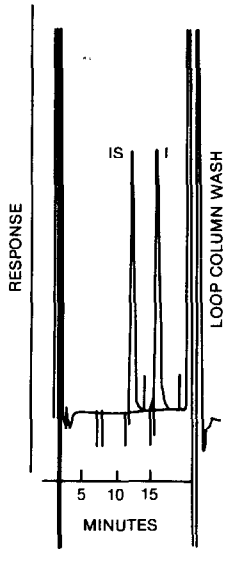

Fig. 2. A chromatogram of sulconazole nitrate (I) cream sample obtained by the automated method. Mobile phase, acetonitrile–water–acetic acid (48:51:1), 0.01 *M* $KClO_4$; flow-rate, 1.5 ml/min; loop column, Partisil-10-ODS-3, 10 μm; analytical column, Partisil-10-ODS-3, 10 μm; detection at 236 nm and 0.04 a.u.f.s.

TABLE IV

LINEARITY AND ACCURACY OF AUTOMATED METHODS

Compound	*Linearity*			*Accuracy*	
	Slope ± 95% confidence limits	*Intercept ± 95% confidence limits*	*Correlation coefficient*	*Mean recovery (%)*	*Relative standard deviation (%)*
Sulconazole nitrate	0.9859 ±0.028	0.044 ±0.120	0.9997	98.2	1.1
Triple corticoid					
Fluocinonide	1.017 ±0.032	−0.004 ±0.013	0.9996	100.5	0.7
Procinonide	1.012 ±0.046	−0.002 ±0.008	0.9992	99.9	1.6
Ciprocinonide	0.9991 ±0.033	−0.0001 ±0.003	0.9996	100.1	1.6

significant effect on the chromatographic parameters (Table III). The excellent linearity and accuracy results are presented in Table IV. The precision of the automated method was compared with the manual liquid/liquid extraction method by two analysts on two days. The results in Table V indicate that the reproducibilities of the two methods are excellent and comparable.

TABLE V

ASSAY RESULTS USING THE AUTOMATED METHOD AND A MANUAL LIQUID/LIQUID EXTRACTION METHOD FOR SULCONAZOLE NITRATE IN A CREAM FORMULATION

	Analyst 1		*Analyst 2*	
	*Automated method % LS**	*Manual** method % LS*	*Automated method % LS*	*Manual** method % LS*
Day 1	102.7	103.1	103.1	102.9
	102.7	102.1	103.0	102.7
	102.3	102.1	103.8	102.0
Day 2	102.5	103.3	102.6	102.5
	104.0	104.8	100.6	103.0
	103.3	104.5	101.0	102.0

Mean % LS, automated method = 102.6
standard deviation = 1.0

Mean % LS, manual method = 102.9
standard deviation = 0.9

* LS = Label strength.
** Liquid–liquid extraction.

Analysis of a multicomponent cream

The ability of the automated method to analyze a multicomponent cream was tested using a triple corticoid cream in which the concentrations of the corticoids (fluocinonide, procinonide and ciprocinonide) were low and varied. A stability-indicating liquid–liquid extraction method for the analysis of the triple corticoid cream was described by Shek *et al.*[15].

The automated analysis of the triple corticoid cream was carried out using a Spheri-5 ODS, 5-μm, loop column and an Ultrasphere C_{18}, 5-μm, analytical column. Similar packing material was selected for both columns to preserve the critical resolution between the last two peaks. However, incorporation of the loop column caused a decrease in the plate count (Table III). Fig. 3 is a typical chromatogram of the three corticoids, the major degradation product (fluocinolone acetonide) and the internal standard (norethindrone) as they elute on this sytem. It illustrates that the use of the automated system allows adequate resolution of procinonide and ciprocinonide. Due to the low concentrations of corticoids in the cream, larger sample sizes (5 g) were taken and dissolved in a minimum amount of solvent. The solution was clarified by centrifugation prior to injection onto the system.

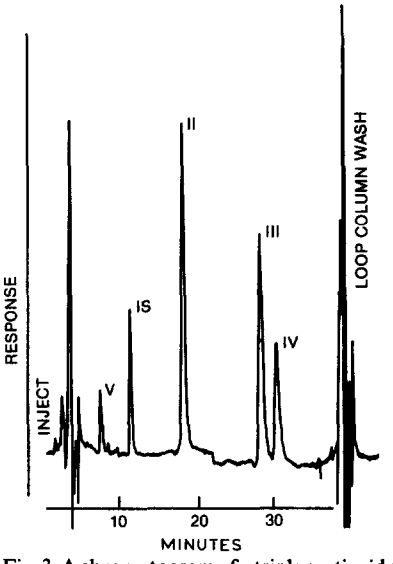

Fig. 3. A chromatogram of a triple corticoid cream sample, fluocinonide (II), procinonide (III), ciprocinonide (IV) and fluocinolone acetonide (V), obtained by the automated method. Mobile phase, THF–acetonitrile–water (4:43:53); flow-rate, 1.0 ml/min; loop column, Spheri-5 ODS, 5 μm; analytical column, Ultraphere ODS, 5 μm; detection at 260 nm and 236 nm, 0.02 a.u.f.s.

The results of the linearity and accuracy studies for this cream are listed in Table IV. Again, the slope and the intercept are not significantly different from one and zero, respectively.

CONCLUSIONS

The results presented in this study indicate that the loop column method can be

automated relatively inexpensively for the simple and rapid clean-up and analysis of drug substances in cream formulations without the need for time-consuming and laborious extractions.

In one case, a slight decrease in chromatographic efficiency was noticed with the use of the loop columns. If tolerated by the system, this is a minor disadvantage when compared with the greatly increased laboratory productivity possible with such an automated system.

ACKNOWLEDGEMENTS

Presented in part at the Pharmaceutical Analysis and Control section, APhA Academy of Pharmaceutical Sciences Western Regional Meeting, San Mateo, California, February 1982.

We thank Dr. Jeremy Higgins of Brownlee Labs for gifts of MPLC™ cartridges, Mr. Thomas Clemow of Autochrom Inc. for help in selection of automation equipment and Dr. E. Shek for helpful discussions.

REFERENCES

1 J. A. Apffel, T. V. Alfredson and R. E. Majors, *J. Chromatogr.*, 206 (1981) 43.
2 D. H. Freeman, *Anal. Chem.*, 53 (1981) 2.
3 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 571.
4 R. J. Dolphin, F. W. Willmott, A. D. Mills and L. P. J. Hoogeveen, *J. Chromatogr.*, 122 (1976) 259.
5 D. D. Koch and P. T. Kissinger, *Anal. Chem.*, 52 (1980) 27.
6 F. Erni, H. P. Keller, C. Morin and M. Schmitt, *J. Chromatogr.*, 204 (1981) 67.
7 H. P. M. van Vliet, Th. C. Bootsman, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 185 (1979) 483.
8 D. A. Burns, *Pharm. Techn.*, 5 (1981) 53.
9 M. C. Harvey and S. D. Stearns, *Amer. Lab.*, 13 (1981) 151.
10 E. J. Benjamin and D. L. Conley, *Int. J. Pharm.*, in press.
11 P. K. Tseng and L. B. Rogers, *J. Chromatogr. Sci.*, 16 (1978) 436.
12 M. Fass, B. Zaro, M. Chaplin and S. Matin, *J. Pharm. Sci.*, 70 (1981) 1338.
13 R. R. Brodei, L. F. Chausseaud and L. M. Walmsley, *J. Chromatogr.*, 155 (1978) 209.
14 J. G. Hoogerheide, S. H. Strusiak, C. R. Taddei, E. R. Townley and B. E. Wyka, *J. Ass. Offic. Anal. Chem.*, 64 (1981) 864.
15 E. Shek, J. Bragonje, E. J. Benjamin, M. J. Sutherland and J. A. P. Gluck, *Int. J. Pharm.*, 11 (1982) 257.