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AN AUTOMATED, COLUMN-SWITCHING HPLC METHOD FOR ANALYZING ACTIVE AND EXCIPIENT MATERIALS IN BOTH CREAM AND OINTMENT FORMULATIONS

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ABSTRACT

We developed a microprocessor-controlled, tandem reverse-phase HPLC method for analyzing both cream and ointment formulations. Analytes included: a developmental corticosteroid in both cream and ointment formulations; a developmental naphthohydroquinone diester in the ointment; and benzyl alcohol, a cream excipient material. Sample preparation consisted simply of dissolving cream or ointment in 30:60 tetrahydrofuran:isopropanol, centrifuging to sediment undissolved residue, and directly injecting onto the HPLC column. With automated switching valves operating in a front-cutting mode, analytes plus internal standard were directed from the reverse-phase precolumn to a 30-cm reverse-phase analytical column while retained excipient materials were backflushed to waste. For comparison, we also analyzed the corticosteroid cream and ointment formulations by reverse-phase HPLC with conventional liquid-liquid partition sample preparation procedures. In all cases, method performance was satisfactory as evidenced by: sample recovery, response linearity, precision, stability specificity, peak tailing factors, peak

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assymetries, peak resolution, and theoretical plate counts. Compared with liquidated extraction techniques, the reported column-switching methods reduced required sample preparation times by a factor of nearly 3.

INTRODUCTION

Topically-applied drugs are frequently incorporated into both hydrophilic cream and hydrocarbon-base ointment formulations.[1] With respect to pharmaceutical analysis, cream and ointment formulations share a common difficulty: both formulation types represent a complex matrix from which active material(s) must be separated prior to analysis. Literature examples of cream and ointment sample preparation techniques mostly require liquid-liquid partitioning to separate active and excipient materials prior to chromatographic analysis.[2-7] The disadvantages of these conventional sample preparation methods are readily apparent. The methods are awkward, tedious, and time-consuming.

High-performance liquid chromatography (HPLC) column-switching techniques afford an obvious alternative to liquid-liquid partitioning for pharmaceutical sample preparation. In general, column-switching HPLC encompasses the use of two columns and appropriate valving to chromatographically: isolate analytes on a precolumn; direct the compounds of interest to a second column; and divert sample matrix constituents to waste. Many specific column-switching HPLC applications are known[8-13] and several reviews have appeared.[14-18] Specifically, with respect to analyzing topical formulations, our laboratory previously described an automated tandem reverse-phase technique for determining steroids and other drugs in vanishing-type creams.[19,20] With this system, the relatively polar analytes rapidly eluted from a 3-cm reverse-phase precolumn onto a 30-cm reverse-phase analytical column while relatively nonpolar excipients were retained on the precolumn and subsequently backflushed with strong solvent to waste. The method satisfied the normal

chromatographic performance criteria and afforded significant time savings compared with conventional extraction techniques.

The demonstrated advantages of the reported column-switching technique notwithstanding, we wished to determine in a practical context, the degree to which we could extend the method to include: 1) additional drugs and excipients incorporated into the same vanishing-type cream and 2) drugs incorporated into an ointment formulation. Accordingly, we have now applied the column-switching HPLC method to analyze the following: 17β-methyl-thiocarbonyl-9-fluoro-11β-17-dihydroxy-16β-methyl androsta-1,4-diene-3-one-17-acetate (timobesone acetate), 1, a developmental corticosteroid; benzyl alcohol, 2, a cream excipient material; and 2,3-dimethoxy-7-chloro-1,4-naphthohydroquinone diacetate, 3, a developmental antipsoriatic. We analyzed compounds 1 and 2 incorporated into a cream formulation similar to that previously described[19,20] and compounds 1 and 3 incorporated into a typical (1) hydrocarbon-base ointment. For comparison, we also analyzed 1 in the cream and ointment formulations by conventional liquid-liquid partitioning workup followed by reverse-phase HPLC. We find that the column-switching HPLC technique satisfactorily provides rapid analysis of all the above drug formulations and simultaneously provides a measure of versatility not found in conventional analysis techniques.

EXPERIMENTAL

Materials

Compounds 1 and 3 were prepared by the Syntex Institute of Organic Chemistry. The purity and structure of 1 and 3 were ascertained by elemental analysis and the usual spectroscopic techniques. Isopropyl alcohol, IPA (Mallinckrodt, Inc.; Paris, KY), benzyl alcohol, 2 (Aldrich Chemical Co.; Milwaukee, WI), acetic acid and petroleum ether (J. T. Baker Chemical Co.; Phillipsburg, NJ) were analytical reagent grade. Tetrahydrofuran THF (Burdick and Jackson Laboratories; Muskegon, MI) and acetonitrile (Baker) were

glass-distilled. Methyl p-hydroxybenzoic acid (MP), fluocinonide (FL), and ethinyl estradiol (EE) plus all cream and ointment excipient materials were U.S.P. grade.

Chromatographic System

The modular HPLC system consisted of: mobile phase pump (Constametric II model, LDC; Riviera Beach, FL) operated at 1.5 mL/min (1500 psi); autosampler (Wisp 71013, Waters Assoc., Inc.; Milford, MA); six-port injection valve (Model 7010 Valve and 7001 Activator, Rheodyne; Cotati, CA) with pneumatic actuator; microprocessor controller (Micromaster WP-6000, Minarik Electric Co.; Los Angeles, CA); solenoid and solenoid interface (Autochrom, Inc.; Milford, MA); uv-visible detector (Model 230, Spectra Physics; San Jose, CA); electronic integrator (Model 4050 printer/plotter with DIM, Spectra Physics); and low-pressure peristaltic pump (Series E, Eldex Laboratories Inc.; Menlo Park, CA). The column assembly consisted of: a reverse-phase precolumn (RP-18 Lichrosorb 10 MPLC^{tm} 3 cm x 4.6mm, Brownlee Laboratories; Santa Clara, CA), a guard column (Co:Pell ODS, 7 cm x 2.1 mm, Whatman, Inc.; Clifton, NJ), and either of two reverse-phase analytical columns (μBondapak C18, 10 μm, 30 cm x 3.9 mm, Waters Assoc., Inc. or ODS-3 RACII, 10 cm x 4.6 mm, Whatman, Inc.)

Methods

In all, six methods were developed for analyzing compounds 1, 2, and 3 in cream and ointment formulations. The methods differed with respect to mobile phase composition, internal standard, and the like. Table I summarizes selected operating parameters for the methods. For Methods 1 and 2, samples were directly injected by the autosampler onto the guard column plus analytical column and the automated column-switching assembly plus precolumn were not employed. For Methods 3 through 6 the column-switching assembly, chromatographic system configuration and automatic operating sequence were as previously described;[19,20] however, in the current study, 75:25 THF:IPA

Table I. Selected Operating Parameters for Analytical Methods 1 Through 6

Method No.	Analyte	Matrix	Analyte Concentration % w/w in Matrix	Sample[a] Preparation	Internal[b] Standard(s)	Mobile Phase $CH_3CN:H_2O:CH_3CO_2H$	Analytical Column	Detector Wavelength, nm	Injection Volume µL
1	1	Cream	0.050	Extraction	FL	45:55:1	RP C18 µBondaPak, 10µm, 30 cm x 3.9 mm	254	100
2	1	Ointment	0.050	Extraction	FL	45:55:1	RP C18 µBondaPak 10µm, 30 cm x 3.9 mm	254	100
3	1	Cream	0.050	Column-Switching	FL	50:50:1	RP C18, µ BondaPak, 10µm, 30 cm x 3.9 mm	254	10
4	1	Ointment	0.050	Column-Switching	FL	50:50:1	RP C18, µBondaPak, 10µm, 30 cm x 3.9 mm	254	10
5	2	Cream	1.0	Column-Switching	MP and FL	28:72:1	RP C18, µBondaPak, 10µm, 30 cm x 3.9 mm	254	10
6	3	Ointment	1.0	Column-Switching	EE	50:50:1	ODS-3, 10µm RACII, 10 cm x 4.6 mm	280	10

[a]See experiemental section for details of liquid-liquid extraction sample preparation methods. In methods 1 and 2, the precolumn was eliminated from the chromatographic system and samples were injected directly onto the guard column plus analytical column.
[b]FL is fluocinonide, MP is methyl p-hydroxybenzoic acid, and EE is ethinyl estradiol.

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mixture was substituted as backflush mobile phase, and times for sequenced events were decreased by a factor of approximately two to account for shorter retention times of the analytes.

Methods 1 and 2. Cream and ointment samples were partitioned between petroleum ether and a 75:25 acetonitrile:water mixture containing internal standard. The aqueous layer was removed and the organic layer extracted twice more with of aqueous acetonitrile. The aqueous extracts were combined, diluted with an equal volume of water, and centrifuged 20 min at 2000 rpm prior to assay.

Methods 3 through 6. Cream and ointment samples were dissolved with gentle warming in THF containing internal standard(s). IPA was added, the mixture shaken, and centrifuged as a precaution to precipitate any undissolved residues.

RESULTS AND DISCUSSION

Sample Preparation

The previously described[19, 20] column-switching HPLC method operates in a front-cutting mode to isolate analytes from sample matrix components. We rationalized that the same separation logic would extend to the case of relatively polar drugs incorporated into an essentially nonpolar hydrocarbon-base ointment. It remained, however, to find a suitable solvent mixture that would: 1) readily dissolve cream and ointment samples and 2) not interfere with subsequent reverse-phase chomatographic analysis. We ultimately selected a 30:60 THF:IPA mixture as dissolution and injection solvent for the column-switching chromatographic analysis (Methods 3 through 6, Table I). The THF:IPA cosolvent dissolved essentially all of the cream excipients (and analytes) and dissolved approximately 50% of the ointment excipients (undissolved residual excipients were conveniently removed by centrifugal sedimentation). As described below, the THF content of the

injection solvent did not compromise chromatographic performance providing that injection volumes did not exceed 10 μL. Sample preparation by dissolution in THF:IPA was much less laborious than preparation via conventional liquid-liquid partitioning (Methods 1 and 2). Total sample preparation time (six samples) averaged three hours for Methods 1 and 2 but only 60 min for Methods 3 though 6.

The sample preparation and analysis by Method 5 deserves comment. Our goal was to analyze both compound 1 and compound 2 in cream samples. Because 2 is more polar than 1 we were unable to find a single mobile phase that would separate both compounds from each other, from excipient materials and from the solvent front. To circumvent the separation problem in Method 5, we prepared cream samples with two added internal standards and reduced the mobile phase organic content relative to Method 3. With Method 5, 2 and its internal standard (EE) eluted from the precolumn while 1 and its internal standard (FL) were retained with excipients and backflushed to waste. To assay for 1, we reinjected the same samples, but changed mobile phase to Method 1 content (higher organic fraction), thereby removing 2 and EE with the solvent front and simultaneously separating 1 and FL from the solvent front and from excipients.

To demonstrate the utility of the column-switching HPLC technique we compared Methods 1 through 6 with respect to accuracy, precision, response linearity, and specificity. The following section summarizes these results.

Analytical Statistics

For each method we prepared (in duplicate) spiked placebos corresponding to 70, 80, 90, 100, 110 and 120% of the sample analyte concentrations listed in Table I (i.e. 0.05% w/w for Methods 1 through 4 and 1.0% w/w for Methods 5 and 6). To demonstrate that peak broadening in sample versus standard determinations did not influence analyte quantitation, we quantitated the analytes by both peak height and peak area measurements. For each spiked

placebo we determined the analyte concentration and calculated percentage recovery: % recovery = $100 \cdot [\text{Analyte}]_{\text{observed}} \div [\text{Analyte}]_{\text{added}}$.

We averaged the recovery data for each method to obtain mean recoveries and the standard deviation (SD) about each mean. Table II lists the mean recovery values. We statistically compared peak height versus peak area recovery means (paired t test, α=0.05/2) and peak height versus area variances (F test, α=0.05/2) to identify potential limitations in the quantitation methods. Table II shows that height versus area means and variances differed significantly for some, but not all methods. In all cases, recovery means were acceptably close to 100%.

Table II also provides precision data for Methods 1 through 4. Here, for each method, we assayed twelve different samples made to approximately 100% of the listed (Table I) analyte concentration and calculated mean recovery ($\pm$SD) values. Again we statistically compared peak height versus peak area means and variances. The precision data showed recovery means to be statistically different for Methods 1 and 2 but not for Methods 3 and 4. For Methods 1 through 3, there were no statistically significant differences among the recovery variances, and all four methods gave relative standard deviations <1.5%.

To demonstrated method response linearity, we used the spiked placebo recovery data (at 70 to 120% of tested analyte concentration, see above) and least-squares linear regression analysis according to the following expression: $[\text{Analyte}]_{\text{observed}} = \text{Intercept} + \text{Slope} \cdot [\text{Analyte}]_{\text{added}}$.

All six methods were highly linear as evidenced by: slope and intercept values equaling, respectively, unity and zero (at the 95% confidence level); correlation coefficients greater than 0.9996; and standard errors of regression less than 3.2.

Table II. Percentage Recovery and Precision Statistics for Cream and Ointment Analyses

Method[a]	Recovery Mean % ± SD Peak Height	Recovery Mean % ± SD Peak Area	Recovery Precision, % ± SD Peak Height	Recovery Precision, % ± SD Peak Area
1	98.9 ± 0.40[c]	98.3 ± 3.6[c]	101[b] ± 0.45	102[b] ± 0.76
2	99.7 ± 1.1[c]	102 ± 2.7[c]	99.8[b] ± 0.75	98.6[b] ± 0.82
3	97.8[b] ± 0.85	99.7[b] ± 0.99	103 ± 1.2	103 ± 0.94
4	99.6 ± 0.97	99.9 ± 0.84	99.3 ± 0.45[c]	99.9 ± 1.4[c]
5	101[b] ± 0.98	100[b] ± 1.2	d	d
6	99.8 ± 0.92[c]	100 ± 0.46[c]	d	d

[a]See Table I for method description
[b]Peak height versus peak area means statistically different (t-test, $\alpha = 0.05/2$).
[c]Peak height versus peak area variances statistically different (F-test, $\alpha = 0.05/2$).
[d]Not determined.

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In summary, all six methods met the normal statistical acceptability criteria for linearity, accuracy, and precision. An additional criterion (in cases where the methods will apply to drug product stability monitoring) is method specificity. The paragraph below discusses this aspect of method acceptability.

Representative Chromatograms

For Methods 1 through 6, placebo chromatograms showed no interferences with analyte, internal standard or analyte degradation products. For this reason we do not present placebo chromatograms. Figures 1 through 3 show representative chromatograms for Methods 3, 5 and 6, respectively. In each figure, the chromatogram corresponds to analysis of placebo spiked with analyte plus internal standard plus known or anticipated degradation

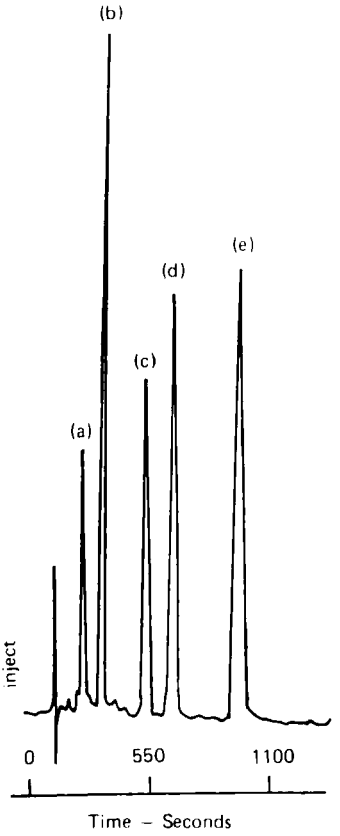

Figure 1. Representative chromatogram of Method 3 showing: degradation products, (a) through (c); FL internal standard (d); and compound 1 (e). Retention times in seconds.

products. Chromatograms for Methods 1, 2, and 4 were indistinguishable from chromatograms for Method 3.

All six methods achieved baseline resolution between analyte, internal standard, and degradation products, thereby demonstrating method stability specificity.

As a final probe of the analytical methods we determined the influence of the precolumn and of injected THF volume on chromatographic performance characteristics. These studies are described below.

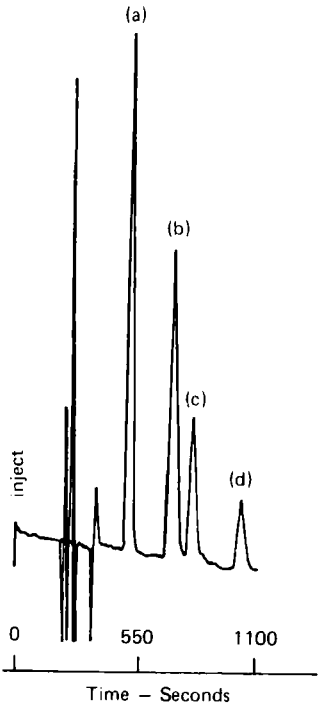

Figure 2. Representative chromatogram of Method 5 showing: compound 2, (a); benzoic acid degradation product, (b); MP internal standard (c); and benzaldehyde degradation product (d). Retention times in seconds.

Chromatographic Performance Characteristics

To demonstrate that the precolumn did not deteriorate analyte chromatography, we determined chromatographic performance parameters for compound 1 and FL internal standard using Methods 1 and 2 (without precolumns) and Method 3 (with precolumn). The selected performance parameters (peak tailing factor, peak assymetry, resolution and theoretical plate count) were calculated according to the usual equations.[16] For each performance parameter, the values obtained with Method 3 were essentially identical to values obtained with Methods 1 and 2 (and for this reason the data are not shown). Thus, in accordance with earlier observations[19,21] we conclude that the MPLC™ precolumn, for all practical purposes, has no negative influence on column-switching chromatography.

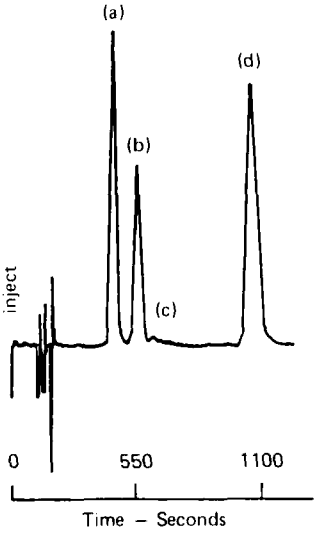

Figure 3. Representative chromatogram of Method 6 showing: EE internal standard, (a); degradation products, (b) and (c); and compound 3, (d). Retention times in seconds.

We investigated the influence of the THF:IPA dissolution and injection solvent on chromatographic performance two ways. First, we prepared reference standard solutions, as well as cream and ointment samples in dissolution solvent containing 20 to 40 percent THF and analyzed the samples by Methods 3 and 4 maintaining a constant 10-μL injection volume. Table III summarizes the results. Secondly, we prepared standard solutions and samples in 30:60 THF:IPA dissolution solvent and assayed by Methods 3 and 4 with 10-to 40-μL injection volumes. Table IV details these data.

Table III shows that for a 10-μL injection volume, dissolution solvent THF content had no significant influence on chromatographic performance within the 20 to 40% THF range investigated. However, Table IV reveals that with 30% THF content in the dissolution solvent, increasing the injection volume beyond 10 μL seriously degraded chromatographic performance. At injection volumes greater than 10 μL, peak fronting occured (as evidenced by decreasing peak tailing factor and assymetry parameters) with concomitant reduction in peak resolution and theoretical plate count.

Table III. Influence of Method 3 and 4 Dissolution Solvent THF Fraction on Chromatographic Performance Parameters[a]

Method[a]	Analyte	THF Fraction %	Tailing Factor		Peak Assymetry		Resolution Factor		Theoretical Plates	
			Standard Solution	Sample	Standard Solution	Sample	Standard Solution	Sample	Standard Solution	Sample
3	FL	20	1.02	1.40	1.31	1.71	5.06	5.05	2770	2790
		32	1.40	1.48	1.32	1.80	5.15	4.99	2840	2640
		40	1.38	1.32	1.55	1.47	5.19	4.99	2790	2770
	1	20	1.29	1.27	1.70	1.55	b	b	2960	2840
		32	1.31	1.31	1.51	1.53	b	b	2940	2880
		40	1.22	1.42	1.23	1.68	b	b	3010	2750
4	FL	20	c	1.26	c	1.40	c	5.05	c	2740
		32	c	1.40	c	1.65	c	5.02	c	2860
		40	c	1.33	c	1.56	c	4.99	c	2790
	1	20	c	1.32	c	1.51	b,c	b	c	2840
		32	c	1.38	c	1.68	b,c	b	c	2770
		40	c	1.39	c	1.69	b,c	b	c	2770

[a]See Table I for method description.
[b]Resolution between FL and 1 shown in row for FL.
[c]Standard solution data identical for Methods 3 and 4.

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Table IV. Influence of Method 3 and 4 Injection Volume on Chromatographic Performance Parameters[a]

Method	Analyte	Injection Volume µL	Tailing Factor		Peak Assymetry		Resolution Factor		Theoretical Plates	
			Standard Solution	Sample	Standard Solution	Sample	Standard Solution	Sample	Standard Solution	Sample
3	FL	10	1.40	1.48	1.32	1.80	5.15	4.99	2840	2640
		15	1.20	1.35	1.31	1.65	5.04	5.05	2700	2770
		25	1.21	1.13	1.27	1.22	4.57	4.23	2040	1820
		30	1.07	0.99	1.17	0.90	4.43	3.83	1880	1340
		40	0.94	0.94	0.81	0.73	3.35	2.98	960	842
	1	10	1.31	1.31	1.51	1.53	b	b	2940	2880
		15	1.31	1.25	1.43	1.45	b	b	2910	2860
		25	1.18	1.15	1.43	1.31	b	b	2510	2110
		30	1.06	1.00	1.11	1.07	b	b	2240	1810
		40	0.93	0.87	0.85	0.76	b	b	1490	1060
4	FL	10	c	1.40	c	1.65	5.15	5.02	c	2860
		15	c	1.33	c	1.50	5.04	5.00	c	2480
		25	c	1.18	c	1.29	4.57	4.65	c	2170
		30	c	0.98	c	0.75	4.43	3.10	c	1160
		40	c	0.83	c	0.58	3.35	2.60	c	766
	1	10	c	1.38	c	1.68	b,c	b	c	2770
		15	c	1.26	c	1.49	b,c	b	c	2970
		25	c	1.29	c	1.46	b,c	b	c	2540
		30	c	0.91	c	0.89	b,c	b	c	977
		40	c	0.93	c	0.60	b,c	b	c	758

[a]See Table I for method description
[b]Resolution between FL and 1 shown in row for FL.
[c]Standard solution data identical for Methods 3 and 4.

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Thus some latitude exists in choosing THF content of dissolution solvent and injection volume. When the total volume of injected THF exceeds 3 µL, however, chromatography performance will noticeably deteriorate.

CONCLUSIONS

The time-saving benefit of column-switching chromatography is reconfirmed here. Compared with conventional liquid-liquid partition sample preparation methods, the column-switching technique reduces sample workup time by a factor of nearly 3.

Another obvious advantage of the column-switching approach, is its versatility. We can now assay both cream and ointment formulations by the column-switching technique, and the methods extend to analytes of widely

disparate structures and retention properties. The case of relatively polar drugs incorporated into formulations containing mostly nonpolar excipients is a common one in pharmaceutical science. Thus the separation that we have employed should have general application.

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