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Note

Use of extraction columns for the isolation of desonide and parabens from creams and ointments for high-performance liquid chromatographic analysis

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Before topical application, corticosteroids are homogenized or dissolved in hydrophilic cream and lipophilic ointment. These formulations represent a complex matrix from which the active component and preservatives must be separated prior to analysis. High-performance liquid chromatography (HPLC) is usually the preferred analytical method, eliminating most interference problems found with other methods such as colorimetric[1] and UV methods[2,3]. HPLC procedures effectively separate the corticosteroids from their most common decomposition products[4,5], other active ingredients and product excipients.

The most common separation methods prior to chromatography were based on liquid–liquid extraction[6,7]. For routine quality control, these methods have several disadvantages. They are time consuming and the extracts often contain residues of the cream and ointment base which may reduce the efficiency of the HPLC column. To overcome these difficulties, extraction methods based on silica columns have been described[8], as well as automated methods based on HPLC combined with column-switching techniques[9–12].

This paper describes an application of disposable Bond Elut™ cartridges for the simultaneous isolation of desonide and parabens from commercial ointments and creams. These compounds can be retained on a silica or on an aminopropyl cartridge when a sample in hexane–chloroform (80:20) is applied to the column. The cream and ointment base are washed out with hexane, and the components then eluted with methanol. The eluate is directly analysed by reversed-phase HPLC. Because of the minimum handling time and ease of operation, the method is well suited for routine quality control.

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EXPERIMENTAL

Chemicals

Analytical grade methanol, *n*-hexane and chloroform were obtained from E. Merck (Darmstadt, F.R.G.). Desonide (particle size: 99% $\leqslant$ 5 μm) was purchased from Sicor (Milan, Italy), methyl hydroxybenzoate and propyl hydroxybenzoate from Nipa Laboratories (Mid Glamorgan, U.K.). Butyl hydroxybenzoate was provided by Sigma (St. Louis, MO, U.S.A.). Desonide creams and ointments (Apolar®) were obtained from Apothekernes Laboratorium (Oslo, Norway). The creams contain 0.1% of desonide and 0.25% of methyl hydroxybenzoate and 0.050% of propyl hydroxybenzoate as preservatives. The ointments contain 0.1% of desonide.

Apparatus

Bond Elut extraction. A Vac Elut vacuum manifold and Bond Elut™ columns from Analytichem International (Harbour City, CA, U.S.A.) were used. The extraction columns were packed with 100 mg of silica, 100 mg diol and 100 mg aminopropyl sorbents (Part. Nos. 601101, 614101 and 611101).

HPLC. The liquid chromatograph was a Model 6000A (Waters Assoc., Milford, MA, U.S.A.) equipped with an SP 8780 XR autosampler (Spectra-Physics, San Jose, CA, U.S.A.) with a 20-μl sample loop, a UV detector Model 440 (Waters Assoc.) with a fixed wavelength of 254 nm and an SP 4270 integrator (Spectra-Physics).

Samples were chromatographed on C_{18} Spheri-5 MPLC™ cartridges (Brownlee Labs., Santa Clara, CA, U.S.A.), and the guard column (30 mm × 4.6 mm I.D.) was directly connected to the analytical column (100 mm × 4.6 mm I.D.). The mobile phase was methanol–water (60:40) and the flow-rate was 1.2 ml/min. The analyses were carried out at ambient temperature.

Liquid–solid extraction procedure

Standard solutions. Stock standard solutions prepared in methanol contained 0.20 mg/ml desonide, 0.50 mg/ml methyl hydroxybenzoate and 0.10 mg/ml propyl hydroxybenzoate. The stock standard solution of butyl hydroxybenzoate as internal standard contained 0.25 mg/ml. The internal standard solution for cream and ointment samples was prepared in chloroform.

Sample solution. Cream or ointment (0.25–0.30) g was accurately weighed into a 25-ml volumetric flask. A 1.00-ml volume of internal standard solution was added and the solution was diluted to the mark in hexane–chloroform (80:20). The solution was gently shaken by a whirlmixer.

Procedure. One Bond Elut silica column for each sample was inserted into the Vac Elut, which was attached to a vacuum source. The washes and waste materials were collected in a vacuum flask placed between the pump and the Vac Elut. With the vacuum on, each column was conditioned with one column volume of hexane–chloroform (80:20). The vacuum was discontinued and 0.50 ml of sample solution were added to each column. The vacuum was reapplied to draw the samples through the columns. The matrix was washed three times with one column volume of hexane.

A 1.5-ml autosampler vial in the Vac Elut autosampler vial rack was placed under each column. With the vacuum off, 0.5 ml methanol were added to each column. After 1–2 min the vacuum was applied to draw the methanol into the autosam-

pler vial, then turned off. The process was repeated with another 0.5 ml of methanol. The solution was gently shaken and the vials were placed in the autosampler for HPLC analysis.

The means and the relative standard deviations for desonide and parabens were calculated after ten separate extractions of cream and ointment samples.

Recovery. Internal standard solution was added to the autosampler vials placed in the Vac Elut rack for determination of the recovery of the extraction procedure. Butyl hydroxybenzoate was used as the internal standard for desonide, methyl hydroxybenzoate and propyl hydroxybenzoate. Desonide was used as internal standard for butyl hydroxybenzoate. The means and the relative standard deviations were calculated after ten assays of the cream and oitment sample extracts on the silica, diol and aminopropyl sorbents.

RESULTS AND DISCUSSION

Sample preparation methods involving liquid–liquid extraction often include one or more time-consuming processes such as heating, cooling and centrifugation. The total preparation time for ten samples is usually 2–4 h, depending on the complexity of the methods. Mobile phases for reversed-phase HPLC analyses or polar solvents such as methanol, acetonitrile or tetrahydrofuran are commonly used as extraction solvents. To minimize the handling time, an attempt was made to prepare the sample solutions directly in lipophilic solvents which dissolve both the active ingredient, the preservatives, the ointment and the cream base. Separation of the compounds of interest from the ointment and the cream base was thereafter achieved on disposable extraction columns. Mixtures of hexane–chloroform were tested as solvents, in combination with disposable extraction columns filled with polar sorbents such as silica, diol and aminopropyl bonded silica. Hexane was used to wash the cream and ointment base from the extraction columns, and the compounds of interest were eluted with methanol.

The chloroform content of the hexane–chloroform mixture was of great im-

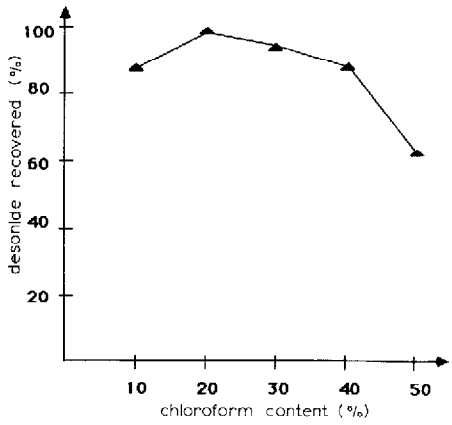

Fig. 1. Recovery of desonide from 0.1% desonide ointment as a function of the composition of the extraction solvent. Each value (▲) is the mean of five determinations.

TABLE I

RECOVERY AND RELATIVE STANDARD DEVIATION (R.S.D.) FOR DESONIDE AND PARABENS EXTRACTED FROM CREAM AND OINTMENT ON SILICA, DIOL AND AMINOPROPYL SORBENTS

Sample	*Silica*		*Aminopropyl*		*Diol*	
	Recovery (%)	*R.S.D. (%)*	*Recovery (%)*	*R.S.D. (%)*	*Recovery (%)*	*R.S.D. (%)*
Cream						
Desonide	99.4	1.0	100.0	4.6	100.5	1.6
Methyl hydroxybenzoate	99.0	0.7	99.3	1.9	93.8	3.0
Propyl hydroxybenzoate	101.8	1.1	100.0	3.4	76.4	7.7
Butyl hydroxybenzoate (internal standard)	99.8	1.2	98.5	2.5	71.0	8.7
Ointment						
Desonide	100.2	1.1	100.5	1.5	94.3	5.6

portance for the recovery. Fig. 1 shows the recovery of desonide from ointment as a function of the chloroform content on the silica column. A low recovery was observed at chloroform concentrations both lower and higher than 20%, while a satisfactory recovery of nearly 100% was achieved with 20% chloroform in hexane. Chloroform concentrations lower than 20% fail to dissolve the ointment base completely. Undissolved ointment base retains desonide and leads to a low recovery. For chloroform concentrations higher than 20% the increased solvent strength of the mixture elutes desonide from the extraction column during sample addition. Hexane–chloroform (80:20) was therefore used as the solvent for sample preparation.

Both desonide and the parabens contain an hydroxyl group and can be retained by hydrogen bonding to polar sorbents. Table I shows the mean recovery and relative

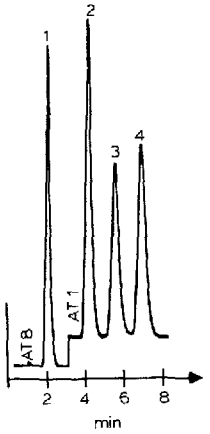

Fig. 2. Chromatogram of a cream extract prepared by Bond Elut extraction. For chromatographic conditions see text. Peaks: 1 = methyl hydroxybenzoate; 2 = propyl hydroxybenzoate; 3 = desonide; 4 = butyl hydroxybenzoate.

TABLE II

DATA FROM CALIBRATION GRAPHS FOR DESONIDE AND PARABENS IN CREAMS AND OINTMENTS

x = Peak area ratio; y = concentration of the sample solution.

Compound	*Calibation graph*	*Correlation coefficient*	*Concentration range (μg/ml)*
Desonide	$y = 12.29x + 0.67$	0.999	2–20
Methyl hydroxybenzoate	$y = 3.42x + 3.00$	0.998	5–50
Propyl hydroxybenzoate	$y = 4.08x + 0.61$	0.998	1–10

standard deviation for desonide and parabens from cream and ointment on silica, diol and aminopropyl sorbents. These experiments show that the silica and the aminopropyl sorbents efficiently retained desonide and parasbens. No losses occurred during the hexane wash, and nearly 100% of desonide and parabens were recovered in the methanol eluate. A virtually complete recovery of desonide was also found on the diol sorbent. The recovery of parabens, however, decreased with increasing alkyl chain length: 93% recovery was observed for methyl hydroxybenzoate and 71% for butyl hydroxybenzoate. These experiments show that both the silica and the aminopropyl columns can be used to process the cream and ointment samples. The silica columns gave, however, the most reproducible recovery for both desonide and parabens. The total sample praparation time for ten samples was 45 min. In our laboratory, silica extraction columns have been used successfully for 1 year in routine quality control of desonide and parabens in creams and ointments.

A typical chromatogram of a cream extract containing butyl hydroxybenzoate as internal standard is shown in Fig. 2. With methanol-water (60:40) as mobile phase, clean chromatograms are obtained and the peaks of interest are well separated. The total time for the HPLC assay was 8 min. Table II gives data for calibration graphs, based on peak area measurements. Table III gives mean values and relative standard deviations for a typical assay of desonide and parabens in cream and ointment. The results presented show that solid-phase extraction is a viable technique for rapid and reproducible preparation of cream and ointment samples for HPLC analysis. Only small amounts of the sorbent and extraction solvent are used for Bond Elut extrac-

TABLE III

MEAN VALUES AND RELATIVE STANDARD DEVIATIONS (R.S.D.) FOR THE ASSAY OF DESONIDE AND PARABENS FROM CREAM AND OINTMENT

Sample	*Compound*	*Cream (n = 10)*		*Claimed content (%)*
		Mean (%)	*R.S.D. (%)*	
Cream	Desonide	0.104	2.0	0.100
	Methyl hydroxybenzoate	0.244	1.2	0.250
	Propyl hydroxybenzoate	0.050	1.4	0.050
Ointment	Desonide	0.098	1.6	0.100

tion, in contrast to the liquid–solid extraction procedure for glucocorticoids published earlier[8]. For high volume assays, on-line sample preparation methods are usually preferred to maximize sample throughput[9–11]. For analysis of smaller series of samples, the Bond Elut procedure is recommended for its versatility and the method is well suited to routine quality control.

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