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Journal of Chromatography A, 828 (1998) 239–246

JOURNAL OF CHROMATOGRAPHY A

Detection of clobetasol propionate as an undeclared steroid in zinc pyrithione formulations by high-performance liquid chromatography with rapid-scanning ultraviolet spectroscopy and mass spectrometry

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Abstract

Clobetasol propionate, an anti-inflammatory glucocorticosteroid, was detected in an over-the-counter topical drug product with no indication on the label of this compound as an ingredient. The product was formulated as a topical spray, a cream, or a shampoo and labeled to contain zinc pyrithione as the active ingredient. The finding of clobetasol propionate in the pharmaceutical products was shown by comparison to an authenticated standard of clobetasol propionate by retention time on normal-phase and reversed-phase HPLC, UV spectroscopy, LC–MS and LC–MS–MS. A simple method was developed and validated for the assay of clobetasol propionate by isocratic reversed-phase HPLC. Several lots contained clobetasol propionate at therapeutic levels of 0.02–0.06%. Zinc pyrithione formulations from two other manufacturers were free of clobetasol propionate.

Keywords: Pharmaceutical analysis; Cosmetics; Clobetasol propionate; Steroids; Zinc pyrithione

1. Introduction

Our laboratory recently detected clobetasol propionate (Fig. 1), an anti-inflammatory glucocorticosteroid [1], in an over-the-counter drug product labeled to contain zinc pyrithione as its active ingredient. This drug product was formulated as a shampoo, a cream, or a topical spray. Clobetasol propionate is not mentioned on the label of these products, and yet, it was found in them at therapeutic levels. These products were advertized and sold via the Internet; they were indicated for the treatment of red, itchy, flaky skin caused by eczema, seborrhea and psoriasis. The active ingredient listed for the cream and the spray was zinc pyrithione at 0.2%; the active ingredients listed for the shampoo was zinc pyrithione at 1% and menthol at 0.25%. Zinc pyrithione is the active ingredient in various over-the-counter shampoos used for the treatment of dandruff and seborrhea of the scalp [1,2]; it is not indicated for the treatment of psoriasis.

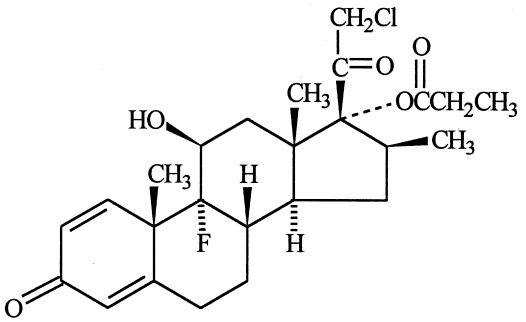

Fig. 1. Structure of clobetasol 17-propionate.

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0021-9673/98/$ – see front matter
PII: S0021-9673(98)00786-9

All corticosteroids have potential side effects [3,4]. The risk of these side effects increases with both long term use and increasing corticosteroid potency, especially if used under no medical supervision. Side effects are caused either by local effects on the skin or systemic effects after the drug is absorbed through the skin. One of the local side effects of the topical corticosteroids is skin atrophy in which the skin becomes thinner and small blood vessels dilate, become visible and appear as a network of red wires [4]. Thinning skin and loss of supportive tissue also leads to skin striae. These atrophic conditions are often permanent. Another local effect may result if topical corticosteroids are used for the treatment of the common form of plaque-type psoriasis, and this treatment is stopped abruptly. In this case, the condition usually becomes much worse and may even convert to pustular psoriasis. Less common but serious systemic effects may result after the drug is absorbed through the skin, particularly with long term use or with potent corticosteroids [3,4]. Percutaneous absorption has an effect on the normal functioning of the hypothalamus, pituitary and adrenal glands and suppresses the production of the bodies own corticosteroids. Some of the systemic side effects of corticosteroids are hypertension, diabetes, Cushing's syndrome, osteoporosis, decreased resistance to infection and growth suppression in children [3,4]. As mentioned above, side effects are more common with more potent steroids. Topical corticosteroids have been categorized into four classes: low, medium, high and very high potency [3]. Clobetasol propionate (0.05%) is in the very high potency class. It is recommended for use for only short durations and on small areas of skin [3]. It is not recommended for children [3] since children are particularly susceptible to side effects of corticosteroids, especially the potent ones. Precautions must be considered before use of topical clobetasol propionate by pregnant women, nursing mothers or older adults [3].

The presence of clobetasol propionate in zinc pyrithione formulations was also shown by the Netherlands Ministry of Health, Welfare and Sports [5] and by the Mayo Clinic [6] using capillary electrophoresis–mass spectrometry. This paper describes the characterization of clobetasol propionate in these drug products by retention times on normal and reversed-phase high-performance liquid chromatography (HPLC), UV spectroscopy, liquid chromatography–mass spectrometry (LC–MS) and LC–MS–MS. It also presents a validated HPLC method for the assay of clobetasol propionate in zinc pyrithione formulations and the results of assays on commercial samples.

2. Experimental

2.1. Instrumentation

2.1.1. LC–UV

The HPLC equipment consisted of a Spectra-Physics Model 8800 ternary gradient pump, Spectra-Physics Model 8880 autosampler, Spectra FOCUS forward optical scanning detector, Compaq Deskpro XL 5100 computer controller with PC 1000 version 3.0.1 software. The analytical column used for reversed-phase chromatography was a Nova-Pak C_{18} column (150×3.9 mm, 4 μm particle size, Waters Associates) with an Inertisil ODS 2 guard column (5 μm particle size, MetaChem SafeGuard). The analytical column used for normal-phase chromatography was a Chromegasphere SI 60 column (250× 4.6 mm, 5 μm, E.S. Industries) with a silica gel guard column (7×3.2 mm, Brownlee NewGuard). The signal was scanned from 200 to 350 nm in order to record UV spectra and monitored at 240 nm. The mobile solvents were acetonitrile–water (1:1) for the reversed-phase system and isopropanol–heptane (1:4) for the normal-phase system. Mobile solvents were filtered through a 0.45-μm nylon membrane filter, degassed by helium sparging, and mixed with a proportionating pump. The flow-rate was 1 ml/min, and the injection volume was 100 μl for the reversed-phase system and 175 μl for the normal-phase system.

2.1.2. LC–MS

The LC–MS equipment consisted of a Waters 600 MS pump and a Finnigan LCQ ion trap mass spectrometer. The column, guard column, mobile phase and flow-rate were as described above for the reversed-phase system. The flow was split such that 5–10 μl/min were passed into the mass spectrometer with the remainder going to waste. The injection

volume was 20 μl. The scan range was 250–900 m/z in MS mode and 130–480 m/z in MS–MS mode. Collisional activated dissociation (CAD) energy was 30% of maximum and the capillary temperature was 200°C.

2.2. Chemicals

Clobetasol propionate was purchased from Sigma (St. Louis, MO, USA). This chemical, defined as 99.5% pure by the Sigma Certificate of Analysis, was further characterized by melting point, optical rotation, high-resolution MS and LC–MS–MS in our laboratories. This substance was used as a reference standard. HPLC-grade acetonitrile, hexane and isopropanol were OmniSolv grade from EM Science (Gibbstown, NJ, USA). Water was purified with the Milli-Q Water System (Millipore, Bedford, MA, USA).

2.3. Standard solution

About 20 mg, accurately weighed, of clobetasol propionate reference standard was placed in a 100-ml volumetric flask, dissolved, and diluted to volume with mobile phase. A quantity of 5.00 ml of this solution was diluted to 200.0 ml with the mobile phase to give a net concentration of 5 μg/ml.

2.4. Sample solutions

Each formulation was shaken well before a composite sample was taken. About 10 g of the zinc pyrithione spray sample was sprayed into a chilled 50-ml Erlenmeyer flask to serve as a sample composite. Similarly, about 10 g of the zinc pyrithione cream or shampoo were placed into a 50-ml Erlenmeyer flask to serve as a sample composite. The composite was mixed to attain a homogeneous mixture and about 200 mg was accurately weighed into a tared 10-ml volumetric flask. The sample was diluted to volume with the mobile phase, shaken vigorously and centrifuged for 5 min. A portion of the clearer supernatant liquid was decanted, filtered if necessary through a 0.45-μm HVLP membrane filter (Millipore), and transferred into a HPLC vial.

3. Results

3.1. Identification of clobetasol propionate by retention time and UV spectra

Analysis of zinc pyrithione spray, cream and shampoo from one manufacturer gave a peak which corresponded in retention time to clobetasol propionate by reversed-phase HPLC. Fig. 2 shows a chromatogram of the cream and clobetasol propionate standard. Normalized UV spectra of these peaks were superimposable. Similarly, in normal-phase HPLC, samples had a peak which corresponded to clobetasol propionate (Fig. 3) and these had superimposable UV spectra. There was a small but distinct difference in the UV spectra of clobetasol propionate in the two mobile solvents, MeCN–water (1:1) and isopropanol–heptane (1:4). An identical spectral solvent effect was observed for the corresponding chromatographic peak from the zinc pyrithione formulations. To further demonstrate that the clobetasol propionate standard and the sample component had identical retention times, each sample was spiked with an estimated equal amount of standard in both reversed-phase and normal-phase mobile solvents. The standard, samples, and spiked samples were then chromatographed in both normal- and reversed-phase modes and peak widths were measured. No increase was seen in the peak width of the spiked sample indicating that the retention times were identical [7].

3.2. Identification of clobetasol propionate by LC–MS and LC–MS–MS

Analysis of the clobetasol propionate standard and zinc pyrithione spray, cream and shampoo samples by RP-HPLC with electrospray mass spectrometry detection gave the same mass spectra. Fig. 4 compares the mass spectrum of clobetasol propionate standard to the spectrum of the corresponding HPLC peak in zinc pyrithione shampoo. Clobetasol propionate has a molecular formula of $C_{25}H_{32}ClFO_5$ and a molecular mass of 466. The peak at m/z 467 is due to the molecular ion [M+H]. The ratio of the relative abundance of m/z 467 to m/z 469 is approximately 3:1 which corresponds to the isotopic ratio of ^{35}Cl to ^{37}Cl confirming the presence of one chlorine atom in the molecule. Also, the MS–MS fragmentation pat-

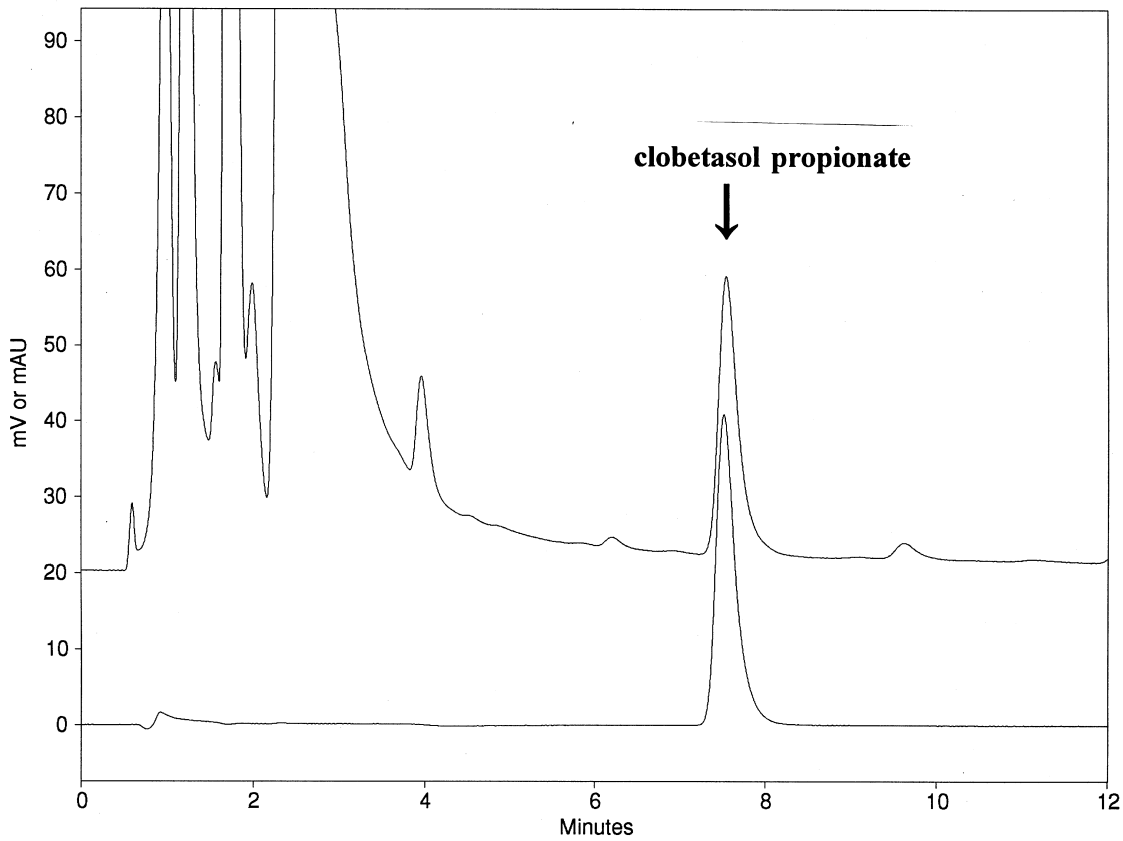

Fig. 2. LC on a C_{18} column with MeCN–water (1:1). Upper chromatogram: Zinc pyrithione cream; lower chromatogram: clobetasol propionate standard.

tern of the molecular ion for standard and sample were identical (Fig. 5).

3.3. Validation of the reversed-phase HPLC method for assay of clobetasol propionate in zinc pyrithione spray, cream and shampoo

3.3.1. System precision

Solutions of clobetasol propionate standard were prepared in the mobile phase at concentrations of 20, 4, 1 and 0.2 μg/ml, equivalent to 0.100, 0.020, 0.005 and 0.001%, respectively, in a 200-mg sample prepared in 10 ml of mobile phase according to the method. Repeated injections of these solutions gave relative standard deviations (R.S.D.s) ($n=6$) for peak areas of 0.18, 0.31, 0.57 and 0.96% at concentrations of 20, 4, 1 and 0.2 μg/ml, respectively. Assays of standard solutions at 5 μg/ml on four separate occasions gave R.S.D.s ($n=6$) of 0.11, 0.09, 0.19 and 0.16%.

3.3.2. Method precision

Samples of zinc pyrithione spray, shampoo and cream, each of which was found to contain no clobetasol propionate, were spiked with clobetasol propionate at concentrations equivalent to 0.001, 0.005, 0.020 and 0.100%. The spiked samples were assayed on three separate occasions. Results are shown in Table 1. The greatest variation was seen in the lowest concentration samples (0.001%).

3.3.3. Linearity

Clobetasol propionate gave a linear response at concentrations of 10, 5, 2, 1, 0.4 and 0.016 μg/ml

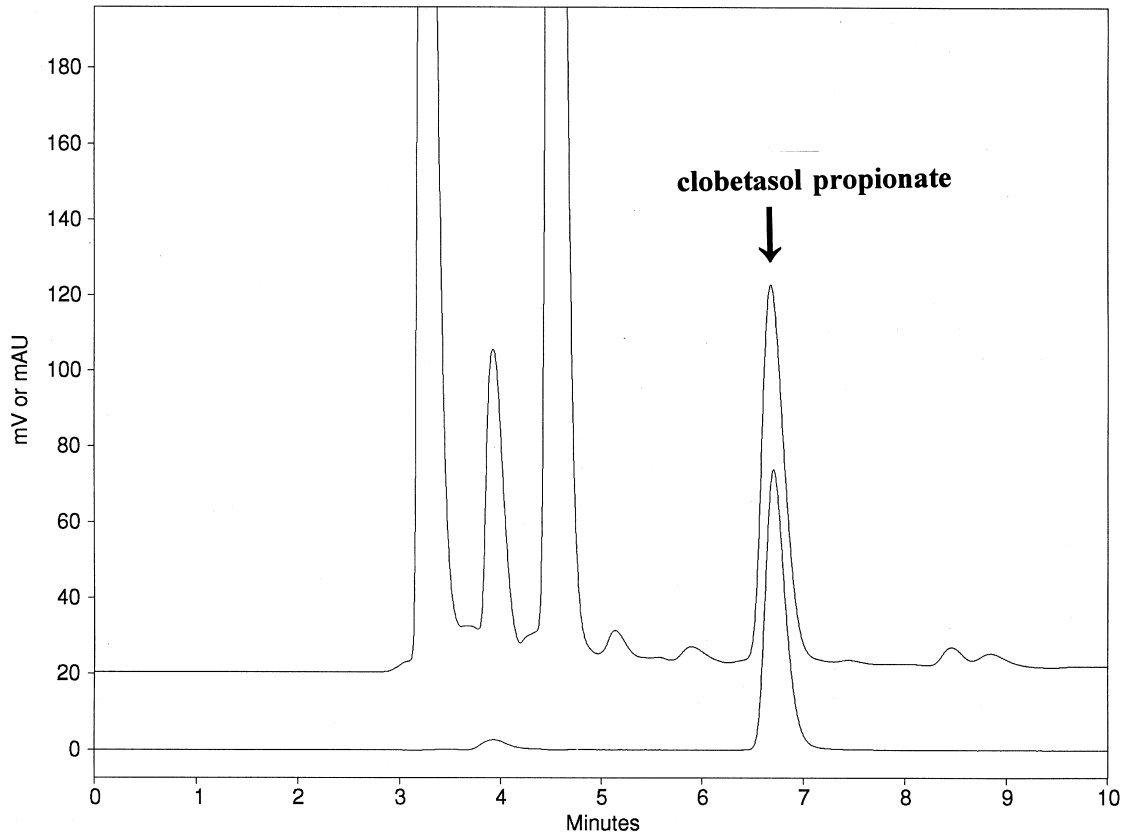

Fig. 3. LC on a silica gel column with isopropanol–heptane (1:4). Upper chromatogram: Zinc pyrithione spray; lower chromatogram: clobetasol propionate standard.

with a correlation coefficient >0.9999. Similarly, samples of zinc pyrithione spray, shampoo and cream, spiked with clobetasol propionate at 0.001, 0.005, 0.020 and 0.100%, each gave correlation coefficients >0.9999.

3.3.4. Limit of detection

The limit of detection for clobetasol propionate standard was determined to be 0.01 μg/ml (signal/noise =3), or 0.00005% clobetasol propionate in a 200 mg sample. A practical limit of quantitation for clobetasol propionate in a sample was estimated to be 0.0005%.

3.3.5. Recovery

Recovery of clobetasol propionate when a sample was spiked with an amount of clobetasol propionate equivalent to 0.025% was 96.9% (0.52% R.S.D., $n=6$) from the spray, 97.0% (0.34% R.S.D., $n=6$) from the shampoo, and 97.8% (0.26% R.S.D., $n=6$) from the cream.

3.3.6. Stability

Samples of zinc pyrithione spray, shampoo and cream were spiked with clobetasol propionate at a concentration of 0.020% and solutions were prepared in the MeCN–water mobile phase. These solutions were assayed when freshly prepared and again after standing for four days at room temperature. Results for the spray, shampoo and cream were 0.0198, 0.0197 and 0.0201%, respectively, for fresh sample solutions, and 0.0198, 0.0196 and 0.0199%, respectively, for four-day old sample solutions. Thus, no decomposition of clobetasol propionate was seen.

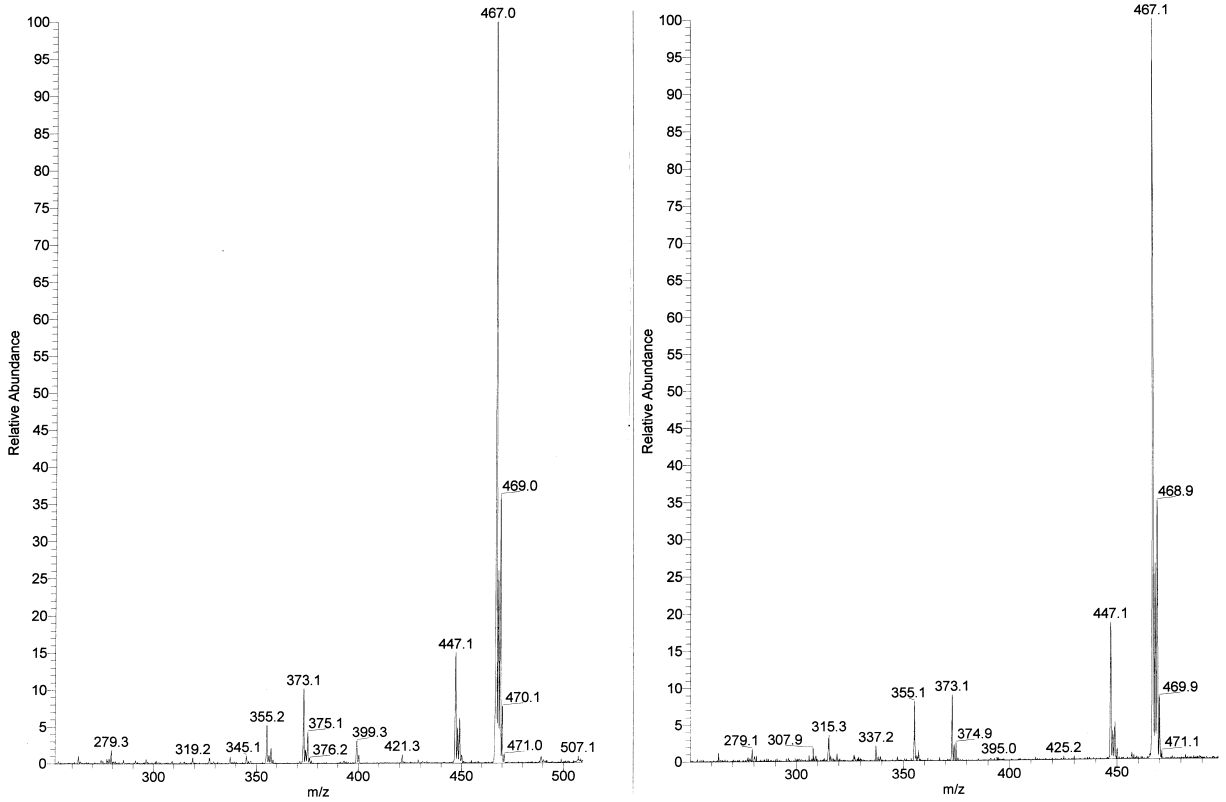

Fig. 4. LC–MS. Mass spectra recorded during reversed-phase chromatography with MeCN–water (55:45). Left: Clobetasol propionate standard, 4.58–4.77 min; right: zinc pyrithione shampoo, 4.56–4.74 min.

3.4. Assay of clobetasol propionate in zinc pyrithione topical products

Early samples were assayed by both normal-phase and reversed-phase HPLC and there was good agreement in assay results from the two modes. Also, matching retention times of clobetasol propionate with the sample in two completely different chromatographic systems provided additional support for identification. However, for convenience, only the reversed-phase HPLC method with UV detection was validated and used for assay.

In the reversed-phase HPLC method the retention time for clobetasol propionate was approximately 7 min. There were numerous components in the spray, shampoo and cream formulations which interfered with subsequent analyses under isocratic conditions. Therefore, after a sample analysis under isocratic conditions for 12 min, the column was flushed with 95% acetonitrile for 16 min to eliminate late eluting sample components, then equilibrated at initial conditions for 20 min. Three clobetasol propionate standard solutions were injected before and after the sample solutions and the mean of peak area/mass was used in the calculations.

Zinc pyrithione topical products from three manufacturers were assayed for clobetasol propionate. The analysis of samples from one manufacturer showed that eight lots (two creams, two shampoos, four sprays) had levels of 0.02–0.06% clobetasol propionate, while five lots (one cream, two shampoos, two sprays) had little or no clobetasol propionate. Interestingly, there were two cases in which two samples with the same lot number contained different concentrations of clobetasol propionate. Two cans of zinc pyrithione spray, both lot L-20, gave assays of 0.03 and 0.05% clobetasol propionate. In another instance, one sample of zinc pyrithione cream, lot

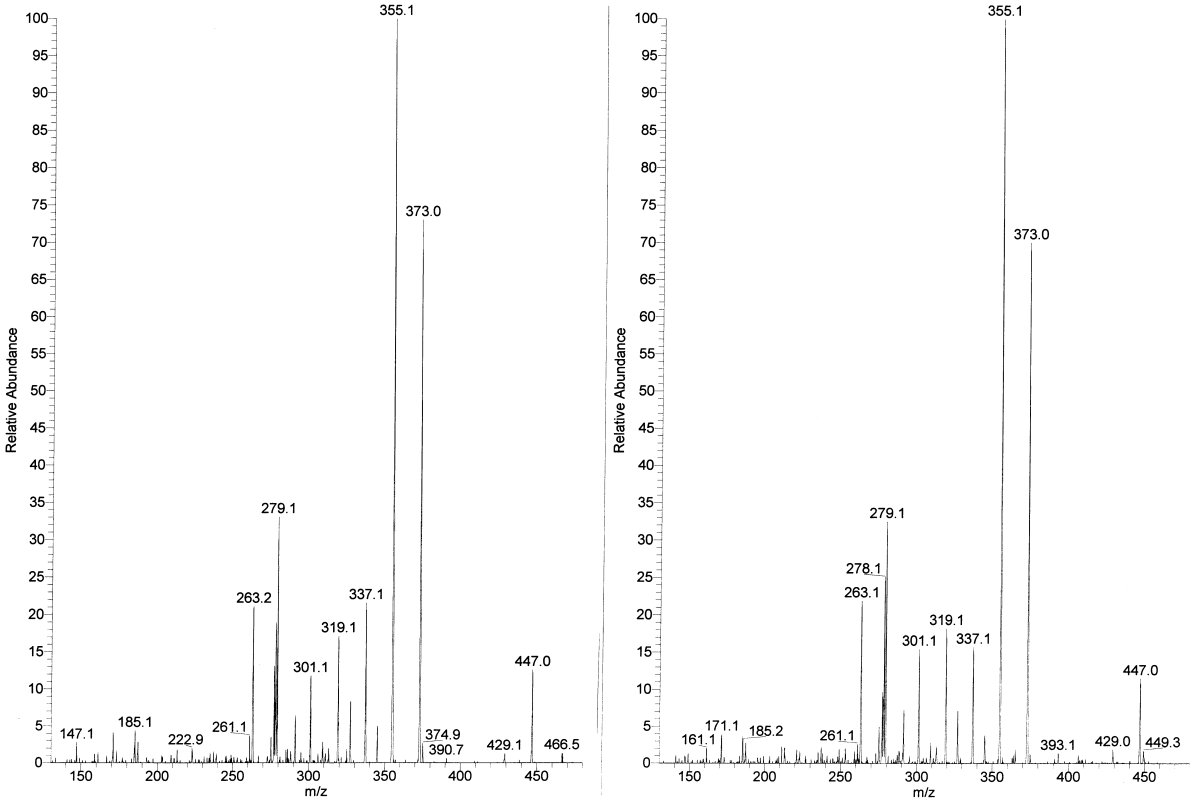

Fig. 5. LC–MS–MS of molecular ion (m/z 467, $[M+H]^+$). Left: Clobetasol propionate standard, 4.59–4.82 min; right: zinc pyrithione shampoo, 4.35–4.86 min.

Table 1
Percent assay of zinc pyrithione spray, shampoo and cream spiked with clobetasol propionate at concentrations of 0.001, 0.005, 0.020 and 0.100%

	Clobetasol propionate concentration (%)			
Day	0.001	0.005	0.020	0.100
Spray				
1	112	102	99	100
2	108	99	98	102
3	108	101	99	96
Shampoo				
1	102	98	98	100
2	96	95	97	99
3	84	95	98	100
Cream				
1	105	102	101	101
2	106	100	99	99
3	104	103	101	100

L-12, was found to contain 0.02% clobetasol propionate, while a second sample of the same lot, received seven months later, contained none. Clobetasol propionate was not found in the zinc pyrithione products from two other manufacturers.

4. Conclusions

The presence of clobetasol propionate in zinc pyrithione spray, cream and shampoo was demonstrated by comparison to an authentic standard of clobetasol propionate by (1) retention time on reversed-phase HPLC, (2) retention time on normal-phase HPLC, (3) UV spectrum in reversed-phase mobile solvent, (4) UV spectrum in normal-phase mobile solvent, (5) co-elution of a mixture of standard and sample containing equal quantities of clobetasol propionate using normal- and reversed-

phase HPLC without peak broadening, (6) LC–MS and (7) LC–MS–MS. A reversed-phase HPLC method was developed and validated for the assay of clobetasol propionate in zinc pyrithione formulations. Clobetasol propionate was found at therapeutic levels of 0.02–0.06% in zinc pyrithione spray, cream and shampoo from one manufacturer. There were two cases in which different samples with the same lot number gave different assay results for clobetasol propionate.

The products under investigation were not approved to contain clobetasol propionate, nor were they approved for the treatment of psoriasis, and they have been recalled from the market as a result of our laboratory findings [8]. Chronic use of a potent topical corticosteroid poses a risk to the patient and should be under the supervision of a physician. Perhaps the greatest risk is posed by the lack of awareness of the presence of a potent steroid; such lack of awareness may lead to improper use unknowingly by a patient, or use by patients for whom it poses a high risk.

Acknowledgements

The authors thank Harry C. Coffman, FDA Division of Testing and Applied Analytical Development, Constance E. Bulawka and Bradford W. Williams, FDA Division of Drug Labeling Compliance, and Martin E. Katz, FDA Florida District Office for their assistance with regulatory and compliance issues. The authors also thank Thomas P. Layloff, Moheb M. Nasr, Henry D. Drew, and Walter L. Zielinski, FDA Division of Testing and Applied Analytical Development, for helpful discussions throughout the study.

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