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A liquid chromatographic method for the determination of tolnaftate in pharmaceutical formulations*

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Abstract: A simple LC method was developed and validated for the analysis of tolnaftate in various pharmaceutical formulations. This method did not require any complex sample extraction procedure. The chromatographic separation was achieved on a reversed-phase, C_{18} column with UV detection at 258 nm. This isocratic system was operated at ambient temperature and required 9 min of chromatographic time. The mobile phase consisted of methanol–aqueous potassium dihydrogen phosphate solution (80:20, v/v) at a flow rate of 1.5 ml min^{-1}. Standard curves were linear over the concentration range of 1.0–51.0 μg ml^{-1}. Within-day and between-day relative standard deviation values ranged from 0.7 to 2.9% and from 1.3 to 3.4%, respectively. This method was used to quantify tolnaftate in microcapsule, microsphere, cream, powder, liquid, liquid aerosol and powder aerosol formulations. This method was also used to study the stability of tolnaftate in solution during its extraction from microcapsule formulations.

Keywords: *Tolnaftate; reversed-phase liquid chromatography; microcapsules/spheres; pharmaceutical formulations; stability.*

Introduction

Tolnaftate is a topical antifungal agent used in the treatment of various dermatophytes. It is available as a crystalline powder (C_{19} H_{17} NOS; M. wt 307.41). The molecular structure of tolnaftate is shown in Fig. 1. It is freely soluble in chloroform, slightly soluble in methanol and practically insoluble in water. Tolnaftate formulations available in the market include: creams, gels, liquid aerosols, powder aerosols, powders and solutions containing 1% (w/w or w/v) of the active drug. Several methods are available for the analysis of tolnaftate. These methods include microbiological [1], spectrophotometric [2–4] and liquid chromatographic (LC) methods [2, 5]. The LC method described in the USP, for the assay of tolnaftate in commercial formulations requires a liquid–liquid extraction procedure prior to analysis and does not include liquid aerosol, microcapsule and microsphere formulations [2]. However, the method reported by Thompson and Carlson did not require any sample extraction steps [5]. When used for the liquid aerosol products, this method found the drug content to be 83–84% (w/w) of the label claim. Both LC methods used acetonitrile and water as the mobile phase system and did not yield satisfactory results when it was attempted to quantitate tolnaftate in microcapsule and microsphere formulations. Tolnaftate microcapsules used in this study were prepared by gelatin–acacia coacervation technique and microspheres were prepared by solvent evaporation method, using ethylcellulose as the polymer material. The objective of this investigation was to develop a simple and accurate LC method to determine the tolnaftate content in currently marketed tolnaftate products and in microcapsule/sphere formulations. This method was also utilized to determine the solution stability of tolnaftate during extraction of the drug from microcapsule formulation.

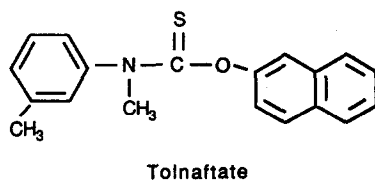

Figure 1
Structure of tolnaftate.

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Experimental

Materials

Tolnaftate (Professional Compounding Centers of America, Houston, TX, USA), tolnaftate reference standard (United States Pharmacopeial Convention, Rockville, MD, USA), tolnaftate powder, cream, liquid aerosol and powder aerosol (Schering-Plough Corp., Memphis, TN, USA), tolnaftate topical solution (Goldline Lab, Ft Lauderdale, FL, USA), 2-naphthol (Janssen Chemical, Turnhoutseweg, Beerse, Belgium), progesterone (Sigma, St Louis, MO, USA), water (HPLC grade), methanol, monobasic potassium phosphate, phosphoric acid, sodium hydroxide (Fisher Chemical, Fairlawn, NJ, USA) were used as received.

Chromatography

The chromatograph comprised a pump (model LC-600) programmed by a system controller (model SCL-6B), an UV–vis spectrophotometric detector (model SPD-6AV) and a recorder (model CR-501), all from Shimadzu (Tokyo, Japan). The separation was carried out on a 250 × 4.6 mm i.d. C_{18} column (Supelco, Bellefonte, PA, USA). The mobile phase was methanol–phosphate buffer (25 mM) (80:20, v/v, apparent pH 6.5) and the flow rate was 1.5 ml min^{-1}. The column effluent was monitored at 258 nm.

Solutions

Phosphate buffer (25 mM). Monobasic potassium phosphate (3.4 g) was dissolved in water (HPLC grade) and the volume made up to 1000 ml.

Mobile phase. Methanol (800 ml) was mixed with 200 ml of phosphate buffer. The solution was filtered through a prefilter and a 0.4 micron polycarbonate filter (Nuclepore, Pleasanton, CA, USA).

Standard solutions. Tolnaftate standard solutions (1.0 to 51.0 μg ml^{-1}) were prepared in mobile phase.

Internal standard solutions. Progesterone solution (113 μg ml^{-1}) was prepared in methanol.

Sample preparation for LC

Internal standard solution (60 μl) was added to a borosilicate culture tube and evaporated to dryness at 40°C in an oven. Standard solution (500 μl) was spiked to the test tube and vortexed for 15 s. An aliquot (20 μl) was analysed by LC.

Data reduction

The ratios of the peak areas of tolnaftate to that of the internal standard were calculated. The unknown tolnaftate concentration was determined from the regression equation relating the peak-area ratio (PAR) of the standards to their concentrations.

Analysis of tolnaftate formulations

Powder. An accurately weighed sample of Tinactin powder (10 mg) was placed in a 50 ml volumetric flask. The volume was adjusted to 50 ml with mobile phase. Approximately 1 ml of the above mixture was filtered through a 0.45 μm Nylon filter (MSI, Westboro, MA, USA) attached to a plastic syringe (Becton Dickinson, Rutherford, NJ, USA). Tolnaftate content in this solution was determined.

Creams. Tolnaftate creams from two different manufacturers (Tinactin, Schering Corporation and Genaspor, Goldline Lab) were used for this study. An accurately weighed amount (0.2–0.25 g) of the cream was transferred into a 100 ml volumetric flask containing 60 ml of mobile phase. This mixture was kept at 40°C for 15 min, sonicated for 2 min and the volume was adjusted to 100 ml with the mobile phase. Tolnaftate content in this solution was then determined after filtration through a 0.45 μm Nylon filter.

Topical solution. A known weight of the topical solution (Goldline Lab) was weighed in to a 100 ml volumetric flask and diluted to volume with the mobile phase and tolnaftate content was determined by the LC method.

Liquid aerosol. The drug content in the liquid aerosol product was determined by two different methods. In case of the first method, the total contents of the aerosol container was carefully discharged into a 250 ml Erlenmeyer flask with intermittent shaking. The propellant was evaporated under nitrogen at 40°C in an analytical evaporator (N-Evap, Organomatic Associates, South Berlin, MA, USA). An aliquot (1 ml) of the solution was transferred into a 100 ml volumetric flask and diluted to

100 ml with mobile phase. The tolnaftate content in the solution was determined after filtration through a 0.45 μm Nylon filter. In the second method, the aerosol container was cooled at −70°C for 1 h and the liquid was transferred quantitatively into a 250 ml Erlenmeyer flask after careful removal of the valve. The rest of the procedure was exactly the same as described in the first method.

Powder aerosol. The total contents of the aerosol container was carefully discharged into a 250 ml Erlenmeyer flask with intermittent shaking and evaporated to dryness under nitrogen at 40°C in an analytical evaporator (N-Evap, Organomatic Associates, South Berlin, MA, USA). An accurately weighed amount (20 mg) of the free flowing powder was transferred into a 100 ml volumetric flask and the volume was adjusted to 100 ml with the mobile phase. The tolnaftate content in the solution was determined after filtration through a 0.45 μm Nylon filter.

Microcapsules. Tolnaftate microcapsules were prepared by the gelatin–acacia coacervation technique. Different drug loaded microcapsules were used for this study. A known amount of the microcapsules (10–12 mg) was mixed with 10 ml 0.1 N NaOH solution in a screw-capped bottle and kept at 50°C for 24 h. This mixture was sonicated for 2 min. The pH of the solution was adjusted to 6.5 with phosphoric acid and the volume was adjusted to 100 ml with the mobile phase. The tolnaftate content in the solution was determined after filtration through a 0.45 μm Nylon filter.

Microspheres. Ethycellulose microspheres containing different drug loads of tolnaftate were prepared by solvent evaporation method. A known amount of microspheres (~10 mg) was weighed in a volumetric flask and 1 ml of methylene chloride was added. After 1 min of sonication, the volume was adjusted to 100 ml with mobile phase. Tolnaftate content in the solution was determined.

Stability of tolnaftate in solution

The stability of tolnaftate in 0.1 N NaOH solution during its extraction from the gelatin–acacia microcapsules was also studied. Known amounts of tolnaftate powder were kept at 50°C in 0.1 N NaOH solution for 24 h. The tolnaftate content in the solutions was determined by the LC method after adjusting the pH to 6.5 with phosphoric acid.

Results and Discussion

Assay characteristics

Specificity. Figure 2 shows representative chromatograms of tolnaftate and the internal standard in mobile phase. No interfering peaks were observed in the chromatograms. Figure 3

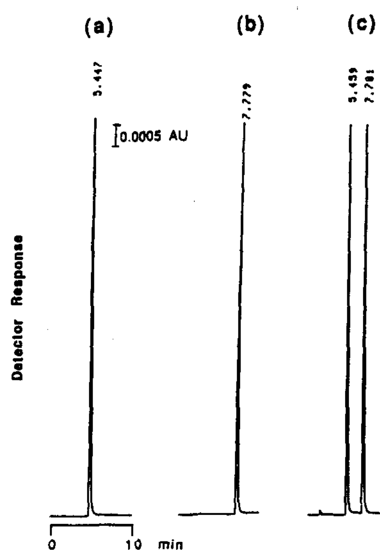

Figure 2
Representative chromatograms obtained following injection of: (a) progesterone (13.4 μg ml^{-1}); (b) tolnaftate (4.3 μg ml^{-1}) and (c) mixture of progesterone (13.4 μg ml^{-1}) and tolnaftate (4.3 μg ml^{-1}).

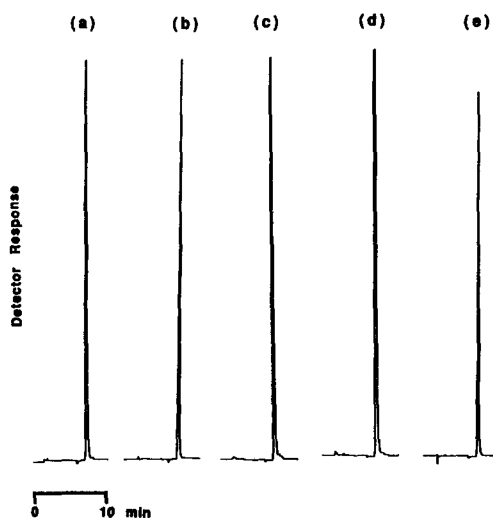

Figure 3
Representative chromatograms obtained following injection of samples prepared from different formulations containing tolnaftate: (a) aerosol liquid; (b) aerosol powder; (c) powder; (d) cream; and (e) ethylcellulose microspheres.

represents chromatograms of tolnaftate obtained after injection of the samples prepared from selected pharmaceutical formulations. None of these chromatograms show any interfering peaks.

Retention time reproducibility. The reproducibility of the retention time of tolnaftate and progesterone was determined from 30 consecutive injections during an analysis of a series of tolnaftate samples. The relative standard deviation (RSD %) was found to be 0.40 and 0.45% for tolnaftate and progesterone, respectively.

Linearity. The standard curves were linear over the concentration range of 1.0–51 μg ml^{-1}. The equation of the standard curve relating the peak area ratio (P) to the tolnaftate concentration (C in μg ml^{-1}) in this range was: $P = 0.164C - 0.012$, $r^2 > 0.999$.

Precision. Within-day precision was determined by analysis of four different standard curves on the same day and all analyses were carried out using the same column. Between-day precision was determined by the analysis of the same solutions on seven different days during a period of 30 days. During this time period, the stock solution was refrigerated (≈4°C) and solutions for the standard curves were prepared fresh each day from the stock solution. The variability in the peak area ratio at each concentration was used to determine the precision of the assay procedure (Table 1). Within-day and between-day RSD values ranged from 0.7 to 2.9% and 1.3 to 3.4%, respectively.

Accuracy. Three quality control samples and the standard solutions were refrigerated for 1 month. The quality control samples were prepared from USP reference standard (Lot: H). These samples were analysed several times during this period and the accuracy of the assay was determined by comparing the measured concentration to its true value (Table 2). The RSD was less than 3%.

Sensitivity. The sensitivity criteria were determined from seven different standard curves using the lowest limit of reliable assay

Table 1
Within-day and between-day analytical precision of tolnaftate assay

Conc. (μg ml^{-1})	Within-day* Peak area ratio‡	Within-day* RSD (%)	Between-day† Peak area ratio§	Between-day† RSD (%)
0.000	0.000	—	0.000	—
1.026	0.177 ± 0.001	0.8	0.176 ± 0.004	2.4
5.130	0.874 ± 0.010	1.1	0.850 ± 0.030	3.4
10.260	1.661 ± 0.048	2.9	1.660 ± 0.020	1.3
20.520	3.291 ± 0.029	0.9	3.370 ± 0.100	3.0
41.040	6.644 ± 0.077	1.2	6.680 ± 0.160	2.4
51.300	8.522 ± 0.104	0.7	8.510 ± 0.130	1.6
Slope	0.164 ± 0.001	0.8	0.166 ± 0.001	0.9

* Analysed on the same day.
† Analysed on seven different days within a period of 30 days.
‡ Mean ± SD; $n = 4$.
§ Mean ± SD; $n = 7$.

Table 2
Accuracy in the analysis of tolnaftate in quality control samples, measured over a period of 30 days

Actual conc. (μg ml^{-1})	Measured conc.* (μg ml^{-1})	Accuracy†	RSD (%)
2.05	2.09 ± 0.04	101.9	2.86
15.39	15.42 ± 0.31	100.2	1.84
30.78	30.95 ± 0.87	100.6	2.60

* Mean ± SN; $n = 5$.
† Accuracy = (measured conc./actual conc.) × 100.

measurement criteria as described by Oppenheimer *et al.* [6]. The critical level is the assay response above which an observed response is reliably recognized as detectable. This was 0.08 ± 0.02 μg ml^{-1} (mean ± SD; n = 7). The detection level is the actual net response which may *a priori* be expected to lead to detection. This was 0.16 ± 0.04 μg ml^{-1}. The determination level, the concentration at which the measurement precision will be satisfactory for quantitative determination, was 0.38 ± 0.11 μg ml^{-1} for a level of precision of 10% RSD.

Applications of the LC method

Analysis of marketed formulation. Tolnaftate content in USP reference standard and in different commercially available formulations was determined. The measured concentrations were compared with the nominal (label claim) concentration (Table 3). The label claim for all the commercial products was 1% (w/w for solid or semisolid and w/v for liquid formulations). The USP Limits for tolnaftate potency are: 98–102% for reference standard; 90–110% for powder and creams; and 90–115% for topical solution [2]. Our results show that the potency of all the products analysed falls within the range set by the USP. However, USP does not have a limit or a method for the analysis of tolnaftate in liquid aerosol product. This LC method was used to determine the drug content in this formulation by two different ways as described in the Experimental section. The results were in good agreement and the RSD was less than 1%. According to the manufacturer, each aerosol liquid container contains 91 mg of tolnaftate [7]. The drug content determined by this method was found to be 87–88% (w/w). Thompson *et al.* [5] also reported 83–84% (w/w) of drug for a similar dosage form using their LC method. Both of these results suggest that the drug might be degrading to some extent in the liquid aerosol formulation or the sample preparation process may not be extracting all of the drug from the formulation. According to the manufacturer [7], the drug is present in a liquid vehicle consisting primarily of ethanol. Therefore, the possibility of incomplete extraction with the mobile phase used in this method is unlikely. The analysis of tolnaftate in the commercial products required a sample filtration step through a 0.45 μm Nylon syringe filter prior to injection into the LC system. The loss of drug if any, during this process was then evaluated. Standard solutions were injected into LC system prior to and after filtration through 0.45 μm Nylon filters. The absolute peak areas of the standard solutions were compared. The results indicated that filtration step does not have any influence on the absolute peak area of the drug.

Tolnaftate content in microcapsule and microsphere formulations. Tolnaftate content in three batches of gelatin–acacia microcapsules and ethylcellulose microspheres containing different drug to polymer ratios were analysed. The measured drug content and the RSD for each microcapsules/spheres are shown in Table 4. The RSD for the microcapsules and the microspheres ranged from 2.6 to 4.0% and 1.0 to 2.8%, respectively.

Stability of tolnaftate

Tolnaftate solution (1% w/v) in polyethylene glycol 400, stored at temperatures ranging

Table 3
Determination of tolnaftate in USP reference standard and various marketed products. The label claim for all these products is 1% (w/w for solid or semisolid and w/v for liquids)

Formulation	Nominal conc. (μg ml^{-1})	Measured conc.* (μg ml^{-1})	Mean† (% nominal)	RSD (%)
USP Ref. standard	12.06	12.21 ± 0.02	101.25 ± 0.68	0.67
Powder	2.01 ± 0.08	2.04 ± 0.06	101.67 ± 1.32	1.29
Cream	2.30 ± 1.73	2.37 ± 1.83	103.04 ± 1.35	1.31
Topical solution	5.44 ± 0.12	5.11 ± 0.16	93.72 ± 2.76	2.94
Aerosol powder	1.97 ± 0.21	1.90 ± 0.28	95.79 ± 3.97	4.14
Aerosol liquid‡	9.10	8.01 ± 0.04	88.00 ± 0.47	0.53
Aerosol liquid§	9.10	7.88 ± 0.03	86.59 ± 0.29	0.33

*Mean ± SD; n = 3.
†The USP limits for tolnaftate potency are: 98–102% for reference standard; 90–110% for powder and creams; 90–115% for topical solution.
‡Determined by method 1; §Determined by method 2.

Table 4
Determination of tolnaftate in microcapsules and microspheres

Formulation	Drug to polymer ratio	Measured* drug content (% w/w)	RSD (%)
Microcapsules	1:1.3	46.49 ± 1.91	4.10
Microcapsules	1:2.0	35.76 ± 1.27	3.55
Microcapsules	1:3.0	28.17 ± 0.72	2.56
Microspheres	1:0.5	64.30 ± 1.77	2.75
Microspheres	1:0.7	61.77 ± 0.60	0.97
Microspheres	1:1.0	47.23 ± 1.27	2.69

*Mean ± SD; $n = 3$.

Table 5
Stability of tolnaftate in 0.1 N NaOH solution (stored at 50°C for 24 h)

Nominal amount (mg)	Measured* amount (mg)	Mean* (% nominal)	RSD (%)
3.10	3.13 ± 0.01	101.23 ± 0.12	0.12
3.30	3.34 ± 0.03	101.21 ± 0.80	0.79
3.10	3.02 ± 0.12	97.52 ± 3.88	3.98

*Mean ± SD; $n = 3$.

from −160 to 100°C, has been reported to be stable [8]. The above solution was also found to be stable when autoclaved under 15 lb of pressure at 120°C for 15 min [8]. Analysis of tolnaftate content in gelatin–acacia microcapsules required the exposure of the microcapsules to 0.1 N NaOH solution at 50°C for 24 h. In order to determine its stability under such condition, known amount of drug was exposed to 0.1 N NaOH solution at 50°C for 24 h and tolnaftate content determined (Table 5). The results indicated that negligible drug was degraded under such conditions and no degradation products were detected in the chromatograms. From the chemical structure of tolnaftate (Fig. 1), it was evident that 2-naphthol might be a possible degradation product. In order to determine its elution behaviour with the parent drug, 2-naphthol was spiked to a tolnaftate solution containing internal standard. The chromatogram (Fig. 4) indicated that 2-naphthol does not coelute with tolnaftate or the internal standard.

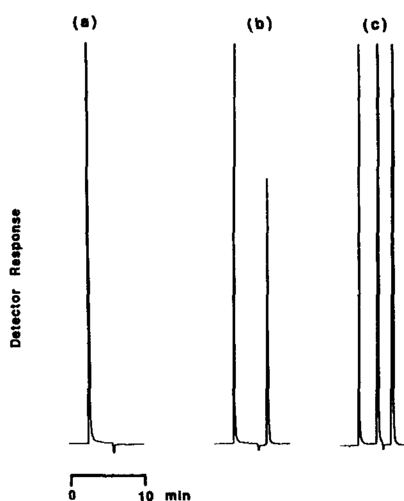

Figure 4
Representative chromatograms obtained following injection of: (a) 2-naphthol; (b) mixture of 2-naphthol and tolnaftate and (c) mixture of 2-naphthol, progesterone and tolnaftate in mobile phase.

Conclusions

A simple, sensitive and reproducible method was developed for the analysis of tolnaftate in all the available marketed formulations. The method did not require any multiple liquid–liquid extraction procedure prior to the LC analysis and used a less expensive mobile phase system as compared to the existing methods. This method was successfully used to determine the tolnaftate content in both gelatin–acacia microcapsule and ethylcellulose microsphere formulations. The stability studies indicated that the amount of tolnaftate degraded during the extraction of drug from gelatin–acacia microcapsules was negligible.

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