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Validation of a Simple and Rapid HPLC Method for Determination of Metronidazole in Dermatological Formulations

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A rapid and simple method using an isocratic high-pressure liquid chromatography (HPLC) and UV detection for the determination of metronidazole in dermatological formulations is presented. Metronidazole samples were extracted with a solution composed of 60% methanol and 40% mobile phase by a procedure that can be completed in less than 10 min. Subsequent separation and quantification was accomplished in less than 20 min using reversed-phase HPLC with isocratic elution with 0.01% trifluoroacetic acid/acetonitrile (85:15%, vol/vol). Validation experiments confirmed the precision and accuracy of the method. When applied to a commercial metronidazole cream and gel formulation, recoveries of 100.4% for cream formulations and 102.3% for gel formulations were obtained. The method should facilitate studies of the formulation compatibility of metronidazole topical formulations with agents that may improve its clinical tolerability for treatment of rosacea.

Keywords metronidazole; HPLC; dermatological formulations

INTRODUCTION

Metronidazole [1-(hydroxyethyl)-2-methyl-5-nitroimidazole] is a cytostatic drug effective for treatment of rosacea, a common chronic syndrome characterized by persistent facial erythema, flushing, edema, pustules, and papules (Pelle, Crawford, & James, 2004; Pye & Burton, 1976). It is available in gel formulation for the treatment of bacterial vaginosis as well as in topical gel and cream for the treatment of inflammatory lesions and erythema of rosacea. Metronidazole is very effective for treatment of rosacea not only after systemic administration but also after topical application (Dahl et al., 1998; Dahl, Jarratt, Kaplan, Tuley, & Baker, 2001). Therefore, it is available in gel and cream formulation in 0.75 and 1.0% that can be applied once or twice daily. Several methods for the determination of metronidazole in plasma either alone or in combination with various drugs have used: spectrophotometric method (Erk & Altun, 2001; Parimoo, Prasad, & Vineeth, 1996; Vega & Sola, 2001), high-pressure liquid chromatography (HPLC) method (Akay, Ozkan, Senturk, & Cevheroglu, 2002; Ali, Chaudhary, & Takieddin, 1999; do Nascimento, Oliveira Ede, & Macedo, 2005; Menelaou, Somogyi, Barclay, & Bochner, 1999; Yeung et al., 1998), and electrophoretic method (Jin, Li, Xu, & Dong, 2000). Methods for determination of metronidazole alone or in combination in pharmaceutical dosage forms (Akay et al., 2002; Baratieri, Barbosa, Freitas, & Martins, 2006) have been reported. The present analytical method for metronidazole determination in the *United States Pharmacopeia* (USP, 2006) applies only to gel formulations, and the method is quite time consuming. To our knowledge, none of these methods have been validated for the determination of metronidazole in dermatological formulations. We report here a method that allows rapid, precise, and accurate determination of metronidazole in dermatological formulations using an HPLC with UV detection. This method should be useful for the determination of metronidazole related to preformulation and formulation studies and facilitate studies of the topical formulation compatibility of metronidazole with agents that improve its clinical efficacy and tolerability for the treatment of rosacea.

MATERIALS AND METHODS

Trifluoroacetic acid was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol (HPLC grade) were purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). Metronidazole, Brij-58, glyceryl monostearate,

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cetostearyl alcohol, white petrolatum, sorbic acid, butylated hydroxytoluene, simethicone, sorbitol 70% solution, propylene glycol, and polyethylene glycol were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). Metronidazole 0.75% Cream (Metronidazole) was from Fougera & Co. (Melville, NY, USA). Metronidazole Gel 0.75% (Vandazole) was from Upsher-Smith Laboratories Inc. (Minneapolis, MN, USA). Double distilled deionized water was used.

HPLC Instrumentation and Conditions

An HPLC system consisting of Varian Pro-star solvent delivery system model 230 (Varian Chromatography Systems, Palo Alto, CA, USA), connected to a UV/Visible Spectroflow 757 absorbance detector (ABI, Mount Holly, NJ, USA) and HP 3395 integrator (Hewlett Packard, Wilmington, DE, USA), was used for detection and separation. The injector was fitted with an injection loop of 50 µL. Chromatographic separations were performed using reverse-phase chromatography using a column of 10µ C18 µBondapak, 300 mm × 3.9 mm (Waters, Milford, CA, USA). The detection wavelength was 315 nm and sensitivity was set at 0.1 a.u.f.s (absorbance units full scale). The mobile phase used was composed of 0.01% trifluoroacetic acid (TFA) and acetonitrile (85:15%, vol/vol) at a flow rate of 0.5 mL/min. The mobile phase was filtered through a 0.45-µm membrane filter (Advantec MFS Inc., Dublin, CA, USA) prior to use.

Preparation of Dermatological Formulations

A metronidazole cream formulation contained the following: water, propylene glycol, sorbitol 70%, sorbic acid, butylated hydroxytoluene, simethicone, white petrolatum, cetostearyl alcohol, Brij-58, glyceryl monostearate, polyethylene glycol, and metronidazole. The ratio of the oil phase to the aqueous phase was (28.5:71.5%, wt/wt). Metronidazole was dissolved in the aqueous phase. The water phase was mixed and placed in one container at 65–75°C in a water bath, and the oil phase was melted and mixed in another container at 65–75°C in the water bath. The oil phase was then added to the water phase and mixed until a cream was formed using an IKA mixer model RW 20DZM (IKA-Works Inc. NC, USA). The cream was cooled to room temperature while stirring.

Extraction of Dermatological Formulations

A sample of 0.2 g of cream or gel was weighed in a 50-mL conical centrifuge tube using a Mettler balance model PB-303S (Mettler-Toledo, Switzerland). The sample was extracted by addition of 30-mL solution composed of 60% methanol and 40% mobile phase followed by vortex mixing for 4 min. An aliquot of 1.5 mL was transferred to a microcentrifuge tube and subjected to centrifugation at 10,000 g for 5 min using an Eppendorf microcentrifuge (Brinkmann Inst Inc., NY, USA). An aliquot of 50 µL of the supernatant was injected directly into the HPLC.

Quantification of Metronidazole

Stock solutions containing 1 mM of metronidazole were prepared in mobile phase and diluted appropriately to provide standards for quantification. The calibration curves were constructed by injecting samples containing metronidazole and determining the peak area at concentrations ranging from 0.005 to 1.0 mM. The peak area was plotted versus the concentration of metronidazole. For the recovery studies, known volumes of metronidazole standard solutions were analyzed and the absolute recovery was calculated by comparing the peak area obtained from cream or gel formulation with the peak area of samples derived from the standard solutions.

RESULTS AND DISCUSSION

Development of Conditions for Rapid Extraction and Separation of Metronidazole from Dermatological Formulations

The desired goal of a method that would allow high throughput quantification of metronidazole in dermatological formulations was to complete an analysis from the formulation in a total of 30 min or less. The extraction procedure developed for metronidazole from dermatological preparations allowed samples to be available for HPLC analysis in approximately 10 min. The extraction solvent mixture composed of methanol and mobile phase (60:40%, vol/vol) was the best to allow no interference with metronidazole peak in HPLC chromatogram. This solvent mixture was chosen based on trial of different type of solvents mixtures. Conditions for a rapid and simple HPLC separation with UV detection were developed using an isocratic elution with a mobile phase composed of 0.01% trifluoroacetic acid (TFA) and acetonitrile (85/15%, vol/vol). These conditions gave well resolved, sharp peaks for metronidazole with retention time of approximately 8.1 min with no interference from the constituents of cream formulation and gel formulation as shown in Figure 1B and C, respectively. Under these conditions, amounts of metronidazole as low as 0.85 µg/mL could be readily detected. With this retention time, sequential analyses could be completed in less than 20 min. As these conditions met our goal for high throughput analysis, validation experiments were completed to determine if the method could achieve the reproducibility and accuracy required for analysis of metronidazole topical formulations.

Method Validation

Specificity

The specificity, defined as the ability of the method to measure metronidazole accurately and specifically in the presence of components in the cream formulations, was determined by analysis of chromatogram of metronidazole extracted from cream formulations. Figure 1, panel A shows a chromatogram for metronidazole dissolved in the mobile phase. Panels B and C show representative chromatograms of metronidazole

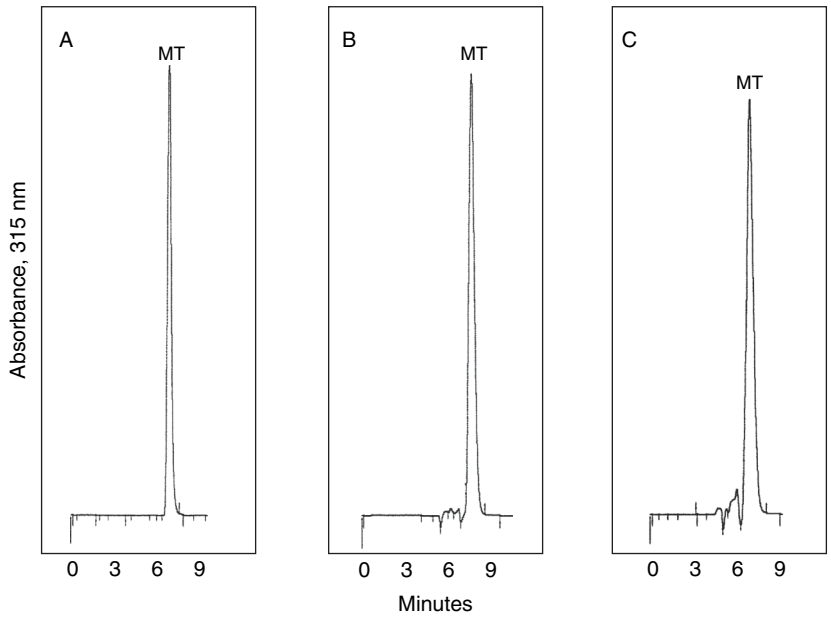

FIGURE 1. HPLC chromatograms for metronidazole (A) dissolved in a mobile phase, (B) extracted from commercial cream formulation, and (C) extracted from commercial gel formulation. The elution of metronidazole is shown as MT.

extracted from cream and gel formulations, respectively. Figure 1 illustrates the ability to detect metronidazole with no interference from the constituents of either cream of gel formulation under these conditions. A placebo cream shows no peaks during the chromatogram run of 10 min. The chromatogram run time of 10 min was sufficient for sample analysis of metronidazole from topical dermatological formulations in less than 20 min.

Linearity and Calibration

The quantification of the method was performed using the peak area of metronidazole. Five standard solutions were prepared and subjected to triplicate analysis by HPLC. The peak area versus concentration was plotted in the concentration range from 0.005 to 1.0 mM to construct the calibration curve. Statistical analysis using least squares regression analysis indicated excellent linearity for metronidazole in the range 0.005–1.0 mM as shown in Table 1.

Accuracy and Precision

The intraday accuracy and precision of the assay was evaluated by analyzing five replicates of cream samples containing metronidazole at three different concentrations as shown in Table 2 The coefficient of variance of the analyzed samples

TABLE 1
Statistical Analysis of Linear Regression of Metronidazole

Run No.	1	2	3	4	5
Concentration (mM)	Peak Area				
0.005	107,565	108,657	108,256	106,856	107,398
0.02	430,224	430,222	433,026	427,424	441,023
0.05	846,312	859,169	844,376	835,391	863,784
0.1	1,482,135	1,460,618	1,492,820	1,492,966	147,698
0.25	3,903,160	3,940,344	3,882,930	3,886,205	392,443
0.5	7,596,190	7,504,416	7,589,066	7,695,088	7,637,528
1.0	15,065,200	15,014,832	15,236,736	14,944,032	15,308,730
Intercept	77,830	81,623	58,399	93,719	62,802
Slope	1.50E + 07	1.49E + 07	1.52E + 07	1.49E + 07	1.52E + 07
R^2-value	.9999	.9998	.9999	.9998	.9999

TABLE 2
Intraday Accuracy, Precision, and Relative Error of Quality Control Samples of Metronidazole

	Theoretical (mg/mL)	Found (mg/mL)	*SD*	CV (%)	Accuracy (%)	RE (%)
Metronidazole	5	5.13	0.10	1.94	102.6	2.60
	10	10.21	0.18	1.76	102.1	2.10
	20	20.33	0.38	1.87	101.7	1.70
Mean					102.1	

$N = 5$.

TABLE 3
Interday Accuracy, Precision, and Relative Error of Quality Control Samples of Metronidazole

	Theoretical (mg/mL)	Found (mg/mL)	*SD*	CV (%)	Accuracy (%)	RE (%)
Metronidazole	5	5.07	0.09	1.77	101.4	1.40
	10	9.98	0.21	2.10	99.8	–0.20
	20	20.46	0.34	1.66	102.3	2.30
Mean					101.1	

$N = 5$.

range from 1.76 to 1.94%, whereas the intraday accuracy of the method ranged from 101.7 to 102.6%. The interday precision of the assay was measured by analyzing replicates of 5, 10, and 20 mg/mL cream samples from day 1 to day 3. Interday accuracy ranged from 99.8 to 102.3%, whereas the interday precision ranged from 1.66 to 2.10% as shown in Table 3.

Recovery

The absolute recovery was calculated by comparing the peak areas of metronidazole standard solutions prepared in a mobile phase to those obtained by extraction of metronidazole from cream formulations at three different concentrations. The results of absolute recoveries of metronidazole creams ranged from 98.9 to 103.3% as shown in Table 4.

TABLE 4
Absolute Recovery of Metronidazole from Cream Formulations

Concentration (g %)	Mean Peak Area		% Recovery
	Mobile Phase	Cream	
0.5	3,129,118	3,094,698	98.9
1.0	6,108,236	6,273,158	102.7
2.0	12,216,472	12,625,027	103.3
Mean	–	–	101.6

$N = 5$.

Application of Method to Metronidazole Commercial Dermatological Formulations

To test the method on commercial products, the absolute recoveries of metronidazole from metronidazole cream (0.75%) and metronidazole gel (0.75%) commercial products were determined as shown in Table 5 The mean recovery from cream formulation was 100.4%, whereas the mean recovery from gel formulation was 102.3%. These results indicate excellent recovery from these topical formulations and no interference from either cream or gel constituents on metronidazole determination under these HPLC conditions.

CONCLUSIONS

The method described here allows simple and accurate determination of metronidazole in dermatological formulation in less than 20 min. The method can be applied to analysis of commercial metronidazole topical formulations. Metronidazole is an important drug that provides benefit to dermatology conditions such as rosacea. Rapid methods of analysis will facilitate preformulation and formulation studies of metronidazole topical formulations and will be useful for studies designed to lead to the discovery of agents that can enhance the efficacy and tolerability of this important therapeutic agent in the treatment of rosacea.

TABLE 5
Absolute Recovery from Metronidazole Cream and Gel Commercial Products

	Concentration (g %)	Mean Peak Area		% Recovery ± *SD*
		Mobile Phase	Cream	
Metronidazole cream	0.75	4,543,677	4,562,411	100.4 ± 1.7
Metronidazole gel	0.75	4,583,655	4,689,249	102.3 ± 2.1

$N = 5$.

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