Metronidazole from Foaming Facial Cleanser using Cleanup by Strata™-X-C SPE Followed by a Rapid Analysis using a Kinetex® XB-C18 Core-Shell HPLC/UHPLC Column

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This technical note describes an effective and thorough SPE cleanup method for metronidazole from foaming facial cleanser. The resulting extract was free of common surfactants found in cosmetics and was analyzed using a 5 minute HPLC method using a Kinetex 2.6µm XB-C18 core shell column.

Introduction

Metronidazole belongs to the drug class of nitroimidazole and is a prescription antibacterial agent used topically to treat acne rosacea.[1] Its formulation strength in lotion, cream, or gel ranges from 0.75-1 %. In the past, the use of 1 % metronidazole gel with different cosmetic regimens was well tolerated.[2]

Although short-term exposure of metronidazole is approved to be safe, nitroimidazoles are known to be mutagenic and carcinogenic.[3] Hence, the use of metronidazole as an active ingredient in cosmetics has been deemed illegal. Due to the regulations placed on metronidazole, a reliable analytical method is in high demand.

Previously reported cosmetic sample preparation methods involve lengthy liquid-liquid extractions.[4-5] In the case of facial cleanser, it is necessary to remove foaming agents in addition to surfactants, lubricants, and other common cosmetic ingredients during the sample preparation process to attain the desired sensitivity of the analytical method as well as to protect the analytical instrumentation from contaminants.

In this study, we report an effective cleanup and highly sensitive analytical method using a polymeric mixed-mode cation-exchange SPE sorbent, Strata-X-C, to extract metronidazole from facial cleanser. The extraction is then followed by a 5 minute LC/MS/MS method using a core-shell Kinetex 2.6 µm XB-C18 HPLC/UHPLC column. The method LLOQ was determined to be 100 pg/mL with good linearity (R^2= 0.9999) over the concentration range of 100 pg/mL to 10 ng/mL. The absolute metronidazole recoveries were 85-92 %.

Materials and Methods

Sample pretreatment:
Facial cleanser samples were pretreated as follows:

1. Dissolve 0.250 g of Foaming Facial Cleanser in 10 mL of 0.1N HCl
2. Vortex until homogeneous
3. Centrifuge sample at 5000 g for 5 minutes

Solid Phase Extraction (SPE)

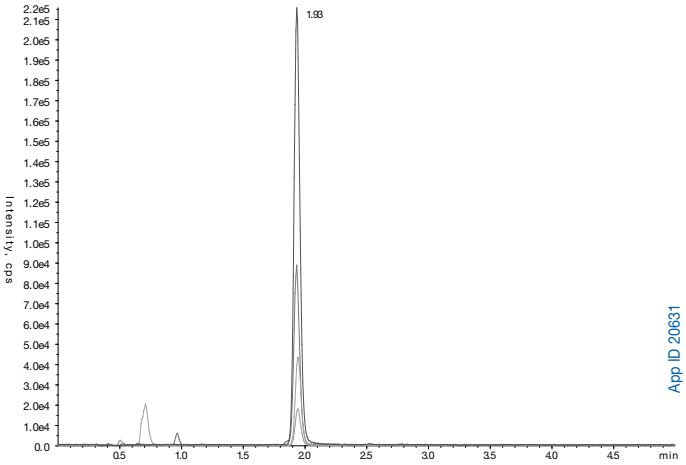
The pretreated sample is further cleaned up and concentrated using SPE.

Cartridge:	Strata-X-C, 30 mg/3 mL
Part No.:	8B-S029-TBJ
Condition:	1 mL Methanol
Equilibrate:	1 mL 0.1N HCl
Load:	3 mL of pretreated sample
Wash 1:	3 mL 0.1N HCl
Wash 2:	3 mL Methanol
Wash 3:	6 mL Ethyl Acetate
Dry:	5 minutes under full vacuum
Elute:	1 mL 5 % NH_4OH in Methanol (v/v)
Dry down:	Evaporate under a stream of nitrogen gas at 50 °C until dry
Reconstitute:	Reconstitute samples with 200 µL of Methanol/0.1 % Formic acid (10:90)

LC/MS/MS

LC/MS/MS was performed using an Agilent® 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 400 bar, equipped with a binary pump, autosampler and an API 4000™ triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA). The ionization source was atmospheric pressure chemical ionization (APCI) run in positive ion mode.

Figure 1.
LC/MS/MS chromatogram of the extracted sample at 100 pg/mL shows effective removal of surfactants



Column: Kinetex® XB-C18 2.6 µm
Dimensions: 50 x 2.1 mm
Part No.: 00B-4496-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: Methanol

Flow Rate: 0.3 mL/min
Temperature: 25 °C
Detection: MS (ambient)
Sample: 1. Metronidazole
2. Metronidazole- $^{13}C_2$, $^{15}N_2$

Gradient:

Time (min)	% A	% B
0.00	95	5
2.00	60	40
3.00	60	40
3.10	95	5
5.00	95	5

Results and Discussion

Foaming facial cleanser consists of various organic acids and their salts, foaming agents, surfactants, lubricants, humectants and colorants. Published work on the determination of metronidazole employs traditional liquid-liquid extraction and fractionation protocols that co-extract many of these matrix interferences with metronidazole, resulting in significant matrix interference in the analysis.[4-5] This is especially true for detection at low levels.

The sp^2-hybridized nitrogen at the 3 position of the imidazole ring in the metronidazole structure is ionizable with a pK_a of about 3.09. At a pH below 2, metronidazole is positively charged and the molecule can be retained via ion-exchange mechanisms on the strong cation-exchange Strata™-X-C SPE sorbent. The benefit of using ion-exchange as a retention mode is that very strong solvent washes can be utilized to effectively remove unwanted matrix interferences away from the analyte of interest without sacrificing analyte recovery.

In this method, following sample loading at low pH, the Strata-X-C SPE cartridge was first washed with acidified water, which removes weakly bound water-soluble matrix interferences. The acidified wash also ensures that metronidazole remains positively charged so that it can strongly interact with the ion-exchange sorbent. Then, a methanol wash was applied to remove hydrophobic matrix interferences that were not solubilized and removed in the previous wash. Finally, a third wash of ethyl acetate was needed to further remove more stubborn interferences such as lubricants, humectants and surfactants.

It is noteworthy to mention that when ethyl acetate was not used, the resulting eluent contained a significant amount of foam, indicating inadequate removal of foaming agents (**Figure 2**)

Figure 2.
The vial on the left shows a significant amount of foam as compared to the vial on the right which is the eluent that resulted after an ethyl acetate wash.

Residual surfactants can retain on the HPLC column too strongly for mobile phase solvents to elute off, causing damage to peak shape, degradation, baseline/retention drift, and signal enhancement/suppression.[6-7] Being able to employ strong washes in this method was extremely valuable in providing a cleaner sample for LC/MS/MS analysis.

With the help of strong washes, the final extract was free of interfering matrix components. The method was found to be linear with an R^2 value of 0.999 over the concentration range from 100 pg/mL to 10 ng/mL in foaming facial cleanser.

Conclusion

The current work describes an effective cleanup and a high throughput analytical method for the determination of metronidazole from foaming facial cleanser. The SPE method utilized a strong cation-exchange sorbent, which allowed for the use of a stronger wash to remove the majority of the matrix interferences. The ability to eliminate matrix interferences to this extent makes detection at low levels possible. This extraction method can also be applied to other types of difficult cosmetic matrices with similar ingredients such as creams and lotions that also require thorough cleanup prior to further analysis.

References

1. Topical Metronidazole Therapy for Rosacea. Bleicher et al. *Arch Dermatol*.**1987**, 123, 609-614
2. *Cutis* 2011, 87(5), 251-9
3. Vet Med Today JAVMA, vol 215. No.1, July 1,1999
4. Determination of Compositions in Cosmetics by Multiple-Instrument. *American Journal of Analytical Chemistry* **2011**, 2, 857-862
5. Determination of common antibiotics and metronidazole in cosmetics by ultra performance liquid chromatography-tandem mass spectrometry. *Chinese Journal of Chromatography* **2009**, 27(1), 50-53
6. Effect of surfactants on surface structure in liquid chromatography. *J Chromatogr Sci* **1987**, 25 (1), 29-32
7. The cleaning and regeneration of reversed-phase HPLC columns. *LCGC Europe*, **2003**, 21, 2-6

APPLICATIONS

Ordering Information

Strata™-X-C SPE

Sorbent Mass	Part Number	Unit
Tube		
30 mg	8B-S029-TAK	1 mL (100/box)
30 mg	8B-S029-TBJ	3 mL (50/box)
60 mg	8B-S029-UBJ	3 mL (50/box)
100 mg	8B-S029-EBJ	3 mL (50/box)
100 mg	8B-S029-ECH	6 mL (30/box)
200 mg	8B-S029-FBJ	3 mL (50/box)
200 mg	8B-S029-FCH	6 mL (30/box)
500 mg	8B-S029-HBJ	3 mL (50/box)
500 mg	8B-S029-HCH	6 mL (30/box)
Giga™ Tube		
500 mg	8B-S029-HDG	12 mL (20/box)
1 g	8B-S029-JDG	12 mL (20/box)
1 g	8B-S029-JEG	20 mL (20/box)
2 g	8B-S029-KEG	20 mL (20/box)
5 g	8B-S029-LFF	60 mL (16/box)
96-Well Plate		
10 mg	8E-S029-AGB	2 Plates/Box
30 mg	8E-S029-TGB	2 Plates/Box
60 mg	8E-S029-UGB	2 Plates/Box

Kinetex® Core-Shell HPLC/UHPLC Columns

2.6 µm Analytical Columns (mm)

Phase	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard™ ULTRA Cartridges* 3/pk
XB-C18	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768 for 4.6 mm ID

2.6 µm MidBore™ Columns (mm)

Phase	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard ULTRA Cartridges* 3/pk
XB-C18	00A-4496-Y0	00B-4496-Y0	00C-4496-Y0	00D-4496-Y0	00F-4496-Y0	AJ0-8775 for 3.0 mm ID

2.6 µm Minibore Columns (mm)

Phase	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges* 3/pk
XB-C18	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782 for 2.1 mm ID

1.7 µm MidBore Columns (mm)

Phase	30 x 3.0	50 x 3.0	100 x 3.0	SecurityGuard ULTRA Cartridges* 3/pk
XB-C18	00A-4498-Y0	00B-4498-Y0	00D-4498-Y0	AJ0-8775 for 3.0 mm ID

1.7 µm Minibore Columns (mm)

Phase	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges* 3/pk
XB-C18	00A-4498-AN	00B-4498-AN	00D-4498-AN	00F-4498-AN	AJ0-8782 for 2.1 mm ID

*SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

APPLICATIONS

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