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Short communication

High-performance liquid chromatographic analysis of mupirocin in polyethylene glycols 400 and 3350 using dual ultraviolet and evaporative light scattering detection

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Abstract

Using dual detection with UV and evaporative light scattering detectors, a gradient LC–MS-compatible method was successfully developed for the separation and identification of impurity and degradates in mupirocin in a complex matrix containing 98% (w/w) of polyethylene glycol (PEG) 400 and PEG 3350. The method enabled the eluent containing the PEGs to be diverted before it entered and contaminated the mass spectrometer source. The impurity was identified as Pseudomonic D, and the degradates were rearrangement product I and rearrangement product II, respectively.

Keywords: Pharmaceutical analysis; Detection, LC; Mupirocin; Polyethylene glycol

1. Introduction

Bactroban Ointment 2% (SmithKline Beecham, King of Prussia, PA, USA) is a dermatologic product indicated for the topical treatment of impetigo due to *Staphylococcus aureus*, β-hemolytic *Streptococcus* and *Streptococcus pyogenes* [1]. Each gram of the ointment contains 20 mg of mupirocin (pseudomonic acid A, Fig. 1) [2,3] in a blend of polyethylene glycol (PEG) 400 and PEG 3350. Mupirocin is produced by fermentation of a strain of *Pseudomonas fluorescens* [4].

To study the degradation of mupirocin in Bactroban Ointment 2%, a mass spectrometry (MS)-compatible HPLC method was required. As PEG 400 and PEG 3350 constitute the bulk (98%) of the sample, the MS-compatible HPLC method must (a) separate the impurities of interests from each other, from the drug substance and from the PEGs, and (b) adequately separate the PEGs to allow eluent containing these PEGs to be diverted before it enters and contaminates the mass spectrometer source. However, the PEGs have no UV chromophore and detection using a refractive index detector with gradient elution is problematic. In this communication, we report on the utility of a Sedex 55 evaporative light scattering detection (ELSD) system connected in tandem to a UV detector for this MS-compatible HPLC method development.

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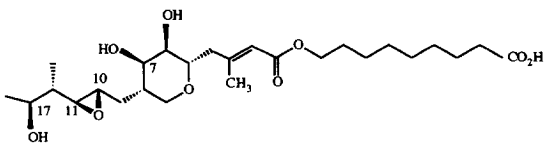

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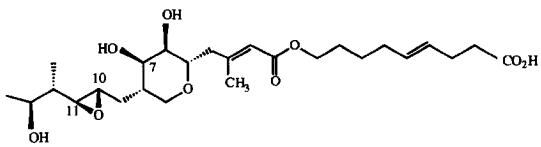

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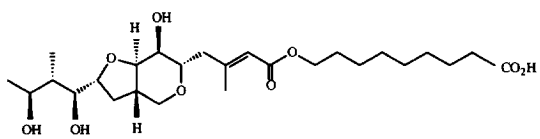
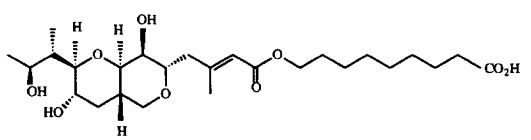


Rearrangement Product I



Rearrangement Product II

Fig. 1. Structures of mupirocin, Pseudomonic D and rearrangement products I and II.

2. Experimental

2.1. Apparatus

Analysis was performed using a modular HPLC system consisting of a Beckman System Gold HPLC pump, a Hewlett-Packard Model 1050 autosampler and variable-wavelength UV detector which was connected in tandem to a Sedex 55 ELSD system (manufactured in Alfortville, France and obtained in the USA through Richard Scientific, Novato, CA, USA). Data were acquired and processed using a Waters 860 networking computer system.

2.2. Chemicals

Bactroban Ointment 2% (stability lot), mupirocin reference standard, rearrangement product I and rearrangement product II were obtained in-house. HPLC-grade ammonium acetate and acetonitrile were obtained from J.T. Baker (Phillipsburg, NJ, USA). Analytical reagent-grade glacial acetic acid was obtained from Mallinckrodt (Paris, KY, USA). Water was filtered through a Milli-Q purification system.

2.3. Chromatographic conditions

All experiments were conducted at ambient temperature (approximately 20°C). UV detection was performed at 240 nm. The temperature of the ELSD nebulizer tube was set at 43°C, the nitrogen inlet pressure at 2.1 bar and the gain setting at 9. HPLC separation was performed on a 25×0.46 cm Zorbax SB C_8 column (Mac Mod Analytical, Chadds Ford, PA, USA). Mobile phase A consists of 100 m*M* ammonium acetate, the pH of which was adjusted to pH 5.7 using glacial acetic acid. Mobile phase B is acetonitrile. Both mobile phases were filtered through 0.45-μm nylon filters and vacuum-degassed prior to use. The mobile phase flow-rate is 0.8 ml/min, and the injection size is 8 μl. The gradient program for the elution is: 3% mobile phase B for 1 min, then linearly increase mobile phase B from 3% to 23% over 23 min, and hold at 23% B for 15 min; then linearly increase mobile phase B from 23% to 31% over 51 min, and hold at 31% B for 10 min; then linearly increase mobile phase B from 31% to 80% over 20 min, and hold at 80% B for 10 min. For reequilibration, decrease mobile phase B from 80% to 3% over 5 min, and hold at 3% B for 15 min.

2.4. Sample preparation

About 2 g of Bactroban Ointment 2% was weighed into a 50-ml glass beaker and dissolved in 10 ml of a 1:1 mixture of mobile phase A and mobile phase B.

3. Results and discussions

Since polyethylene glycol (PEG) 400 and PEG 3350 constitute the majority (98%) of the sample, it was essential that eluents containing these PEGs be diverted before they enter and contaminate the mass

spectrometer source. Although the high content of PEGs facilitated their detection using a refractive index detector, getting a stable baseline with gradient elution which was required for the separation of the degradation products, is problematic [5]. The high background of the mobile phase at low UV wavelength, e.g., at 190–200 nm owing to the high content of ammonium acetate also precludes detection of these PEGs at low UV wavelengths.

ELSD has been used for the detection of non-UV absorbing compounds [6]. We found that UV detection connected in tandem to ELSD is very well suited for this application. The detection of PEGs 400 and 3350 using ELSD and the sensitive detection of the impurity, degradates and drug substance using UV allowed us to develop adequate separations we required for structural identification. The impurity was confirmed by both HPLC spiking experiments and by LC–electrospray MS comparison with authentic compound to be Pseudomonic D, a fermentation by-product. The degradation products were similarly confirmed to be rearrangement product I and rearrangement product II (Fig. 1), respectively. Once the impurity and degradation products were identified, their respective quantity was determined by a validated HPLC procedure using UV detection which provided baseline resolution for all components of interest [7]; area percent was obtained by area normalization. The relative response factors of Pseudomonic D, rearrangement product I and rearrangement product II to mupirocin are all 1:1. The results are shown in Table 1 and the chromatograms for the separation with ELSD and UV detections are shown in Fig. 2a and b, respectively. These degradates were most likely formed from mupirocin via intramolecular attack of the 7-hydroxy group on epoxide carbon C-10 and C-11 with opening of the epoxide ring [8].

Table 1
Impurities, degradates and mupirocin in Bactroban Ointment 2% (stability lot, average of two injections)

	Area (%)	mg/g Bactroban Ointment 2%
Pseudomonic D	3.1	0.6
Rearrangement product 1	3.8	0.8
Rearrangement product 2	6.7	1.3
Mupirocin	86.4	17.3

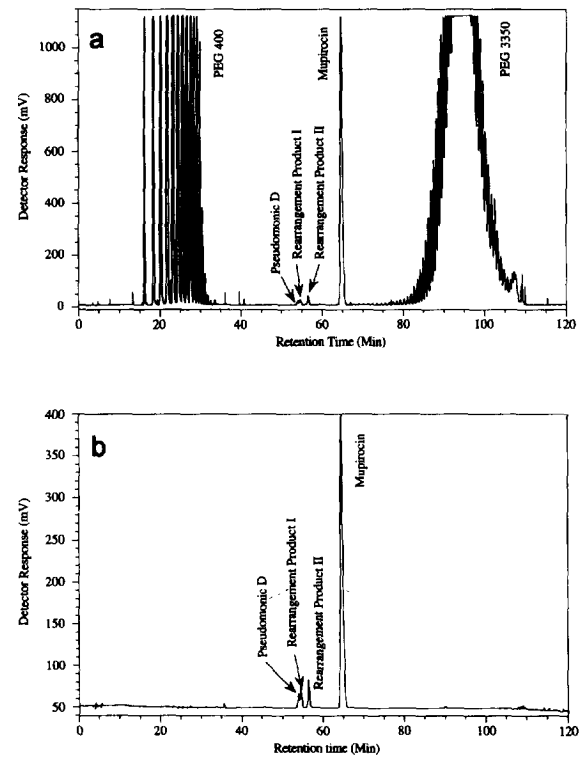


Fig. 2. (a) Chromatogram of Bactroban detection using ELSD. (b) Chromatogram of Bactroban detection using UV at 240 nm.

4. Conclusions

Using dual detection with UV and ELSD, we have successfully developed gradient separation for LC–MS confirmation of impurity and degradates in mupirocin in a complex matrix of PEGs 400 and 3350. The impurity was present at as low as 3% area. These results suggest that dual detection using UV and ELSD in tandem may also have utility in detection for coelution of non-UV absorbing component with an UV-absorbing analyte. This dual detection approach would be especially useful where material balance is an issue.

Acknowledgments

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