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Short communication

Confirmation of ofloxacin precipitation in corneal deposits by microbore liquid chromatography–quadrupole time-of-flight tandem mass spectrometry

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Abstract

We investigated the corneal precipitate of a 6-year-old boy with vernal keratoconjunctivitis (VKC), treated with topical ofloxacin 0.3% eyedrops. Because of the extremely small sample amount (corneal scraping), a very sensitive and specific method was needed with the possibility of an unambiguous identification of ofloxacin, supposed to be present in the precipitate. In this respect, tandem Q-TOF mass spectrometry combined with micro LC (1 mm I.D.) was chosen. Confirmation of the presence of ofloxacin in the deposit was obtained by means of the characteristic product ion spectrum produced by CID. This clearly indicated that the precipitate, removed by corneal scraping from the 6-year-old boy with VKC, contained ofloxacin.

Keywords: Corneal precipitate; Ofloxacin

1. Introduction

Ofloxacin, an antibiotic of the fluoroquinolon family, is frequently used for the treatment of external ocular infections and corneal ulcer. As reported for ciprofloxacin 0.3% and norfloxacin 0.3% topical preparations, corneal precipitation of fluoroquinolones can occur [1–3], due to the formation of the zwitterionic form and thus the lower solubility of this class of drugs near physiological pH. This precipitation has not yet been reported on ofloxacin eyedrops, since its pH–solubility profile is more favorable than the other two [4].

Recently we were confronted with evident corneal deposit formation in two unrelated patients presenting both with vernal keratoconjunctivitis and treated with topical ofloxacin. In view of the known solubility issues of fluoroquinolones we set out to

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investigate the nature of this precipitate. As the patients were treated by scraping of the corneal zone clouded by the precipitate, the treatment simultaneously provided the samples to analyze, i.e., the minute tissue scrapings which were collected. We analysed the corneal deposits of one of the patiënts, a 6-year-old boy, treated with topical ofloxacin 0.3% eyedrops. The sample amount was extremely low, in fact below the lower weighing limit of our analytical balance (10 μg), effectively preventing us from assigning the tissue scraping weight. Consequently, a very sensitive and specific method was needed with the possibility of an unambiguous identification of ofloxacin, supposed to be present in the precipitate.

2. Experimental

2.1. Equipment

A FAMOS, Fully Automated Micro Autosampler from LC Packings, was used for injection of samples and standards. Chromatographic separation was conducted with an Inertsil® ODS-3 C_{18} column (5 μm, 1000 μm I.D.×15 cm; LC Packings, The Netherlands) using an Ultimate Micro Pump HPLC System (LC Packings). Detection was achieved using a Q-TOF mass spectrometer (Micromass, Wythenshawe, UK) in the electrospray positive ion mode (ESI+).

2.2. Reagents and chromatographic conditions

HPLC-grade water, acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Extra pure formic acid was obtained from Sigma–Aldrich (Bornem, Belgium). Ofloxacin and pefloxacin standards were kind gifts of Professor H. Nelis, Laboratory of Pharmaceutical Microbiology, Ghent University, Belgium.

Stock solutions of ofloxacin and pefloxacin were prepared in methanol. Pefloxacin was added in the procedure to provide a qualitative reference in order to substantiate the performance of the analytical measurement. Working standards of ofloxacin were prepared by dilution with water–acetonitrile (90:10), acidified with formic acid to pH 3.0.

Isocratic elution was performed using a flow-rate of 40 μl/min with water–acetonitrile (90/10) brought to pH 3.0 with formic acid. All of the column effluent was directed into the mass spectrometer.

2.3. MS conditions

The Q-TOF instrument was operated in the MS–MS mode using argon as collision gas. TOFMS resolution was approximately 5000 (FWHM). MS–MS data were acquired over a m/z range of 50–400. Source tuning parameters were optimised for sensitivity using continuous ofloxacin infusion. Optimal sensitivity was achieved with an ESI capillary voltage of 2900 V and a cone voltage of 40 V. Collision energy also needed optimisation. To that end, a mix of pefloxacin and ofloxacin was repeatedly injected while stepwise varying collision energy from 10 to 40 eV. Based on the resulting collision-induced dissociation (CID) spectra, an optimal value of 28 eV for ofloxacin and 26 eV for pefloxacin was found.

2.4. Sample pre-treatment

The corneal deposits were surgically removed from the 6-year-old boy by scraping, 7 days after the last topical treatment with ofloxacin eyedrops. To this very limited amount of sample, 100 μl acetonitrile was added. For optimum analyte extraction it was first placed in an ultrasonic bath for 1 h and then overnight in a lab shaker. Subsequently, 150 μl of water, acidified with formic acid to pH 3, the pH at which the zwitterion presents highest solubility, was added. Pefloxacin was added and the mixture again placed in the lab shaker for some time. After centrifugation, 10 μl was injected in the LC–MS–MS system.

3. Results and discussion

3.1. Analytical performance

In order to substantiate the reliability of the, in essence, qualitative procedure, we chose to introduce pefloxacin, because of its structural similarity to and small mass difference with the analyte, ofloxacin, as well as a limited number of method performance checks.

Identity confirmation of ofloxacin was based on a combination of relative retention time and the full scan spectrum (MS–MS) which a Q-TOF instrument, in view of its non-scanning mass analyzer, is able to produce for very low concentrations.

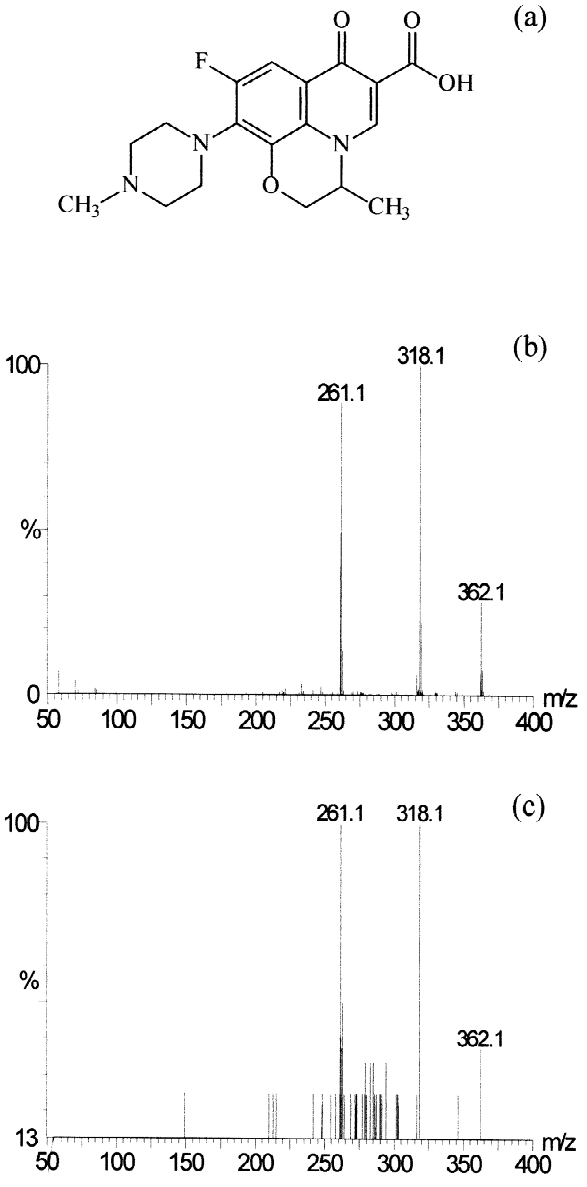

Fig. 1. Determination of ofloxacin. (a), molecular structure of ofloxacin. (b), MS–MS spectrum of ofloxacin standard and (c) MS–MS spectrum of ofloxacin in the corneal deposits.

Summation of the protonated molecular ion $[M+H]^+$ and the two most intense product ions (m/z 261.1+318.1+362.1 for ofloxacin and m/z 233.1+290.1+334.1 for pefloxacin) affords a selective, reconstructed ion fragmentogram.

3.2. Sample findings

The reconstructed ion fragmentogram obtained for the clinical sample showed two peaks which could be identified as pefloxacin ($t_R = 21.06$ min) and ofloxacin ($t_R = 18.44$ min). Although, as expected, the amount of ofloxacin found in the minute tissue shred was very low, a diagnostic CID product ion spectrum (prominent peaks: m/z 362.2 $[M+H]^+$; 318.1; 261.1) was obtained (Fig. 1) which, in combination with the relative retention time, allowed unequivocal confirmation of the presence of ofloxacin in the corneal scraping. As such, we were able to fulfill our primary goal, confirmation of ofloxacin presence in the precipitate.

From a clinical point of view, one must consider the pharmacokinetic profile of ofloxacin and the medical history of the patient. Ofloxacin is rapidly eliminated from the body (plasma $t_{1/2}$ is 6–7 h), thus there is complete elimination after 1.5 days. Despite the fact that tissue will have retarded release of the drug, especially in the cornea where perfusion is limited, one should compare the comparatively fast elimination of ofloxacin with the time interval between cessation of topical treatment and corneal scraping of 7 days. We conclude that depot formation of ofloxacin through precipitation is the basis for this prolonged presence.

4. Conclusion

We were able to demonstrate ofloxacin in a corneal precipitate by optimising sensitivity i.e., through the use of micro LC, and by taking advantage of the outstanding full-scan sensitivity of a TOF analyser.

In contrast to other fluoroquinolone members such as ciprofloxacin, no reports have as yet been published on ofloxacin precipitation after ocular topical

use. Our findings strongly suggest that clinicians should be aware that, at least in the treatment of VKC associated ulcers, deposits can occur after the use of ofloxacin too.

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