Analysis of Ofloxacin Corneal Deposits by Microbore HPLC – Tandem MS with Electrospray Ionisation

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1. Introduction

Nowadays, ophthalmic formulations of new antibiotics of the fluoroquinolon family, including ofloxacin, are frequently used for the topical treatment of external ocular infections and corneal ulcer. Despite of the safe character of this class of drugs, a white crystalline deposit has been reported for ciprofloxacin and norfloxacin. At the moment, this precipitation has not yet been reported on ofloxacin eyedrops. In this case 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, provided by 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 was chosen.

2. Aim

Development of an, in essence, qualitative procedure to positively indentify ofloxacin in the corneal deposits. Nevertheless, the method was extended with a quantitative dimension, to substantiate the amount of ofloxacin in the precipitate.

3. Materials and methods

HPLC Conditions:

- Column: Inertsil® ODS-3 C18 (5µm, 1000 µm I.D. x 15 cm; LC Packings)
- Mobile Phase: 90/10 water/acetonitrile brought to pH 3.0 with formic acid; isocratic elution, 40 µl/min.
- HPLC: Ultimate Micro Pump HPLC System (LC Packings)
- Autosampler: FAMOS (LC Packings), 10 µl injection

MS Conditions:

- Mass Spectrometer: Micromass Q-TOF hybrid mass spectrometer
- Ion Source: orthogonal electrospray source (Z-spray®) in positive ion mode
- Capillary voltage: 2900V
- Cone: 40V
- Collision energy: 28 eV for ofloxacin, 26 eV for pefloxacin

Pefloxacin was chosen as internal standard, because of its structural similarity to and small mass difference with the analyte, ofloxacin. Working standard solutions of ofloxacin were prepared in the concentration range 0.5 µg/L–5 mg/L by dilution with 90/10 water/acetonitrile, acidified with formic acid to pH 3.0. The internal standard was present in a final concentration of 0.5 mg/L. The injection parameters were optimised in the microliter pick up mode, meaning that exactly 10 µl of the samples could be injected, without any sample loss. This method is particularly suited for limited sample volumes, as in our case of the extremely restricted quantity of low dosed corneal deposits. We opted specifically for Micro LC (1 mm ID) because of the improved sensitivity obtained by this approach. The Q-TOF instrument was operated in the MS/MS mode, selecting both protonated molecular ions *m/z* 362.1 for ofloxacin and *m/z* 334.1 for pefloxacin. Qualitative identity confirmation of ofloxacin was based on a combination of relative retention time and the full scan product ion spectrum, which a Q-TOF is able to produce for very low concentrations.

To the corneal deposits, surgically removed from the 6-year old boy by scraping, 100 µL acetonitrile was added. After ultrasonication, it was placed overnight in a lab shaker. Subsequently, 150 µL of water, acidified with formic acid to pH 3.0, together with the internal standard pefloxacin, was added. Finally, 10 µL was injected in the LC-MS/MS system.

4. Results and discussion

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) permitted the construction of linear response curves (between 0.5 µg/L or 5 pg on column and 5 mg/L, respectively, 50 ng on column) with correlation coefficients between 0.9991 and 0.9994. The limit of detection (retaining full scan spectral identity confirmation potential) was 0.4 µg/L and the limit of quantitation 0.5 µg/L. The reconstructed ion fragmentogram obtained for the clinical sample showed two peaks which could be identified as the internal standard pefloxacin (t_R = 21.06 min.) and ofloxacin (t_R = 18.44 min.) (Fig.1) . A diagnostic CID product ion spectrum (prominent peaks: m/z 362.2 $[M+H]^+$; 318.1; 261.1) was obtained (Fig.2) which, in combination with the relative retention time, allowed unequivocal confirmation of the presence of ofloxacin in the corneal scraping. Relating the sample peak area ratio to the standards, revealed an ofloxacine concentration of 0.5 µg/L. As such, we can substantiate the presence of at least 125 pg of ofloxacin in the corneal scraping.

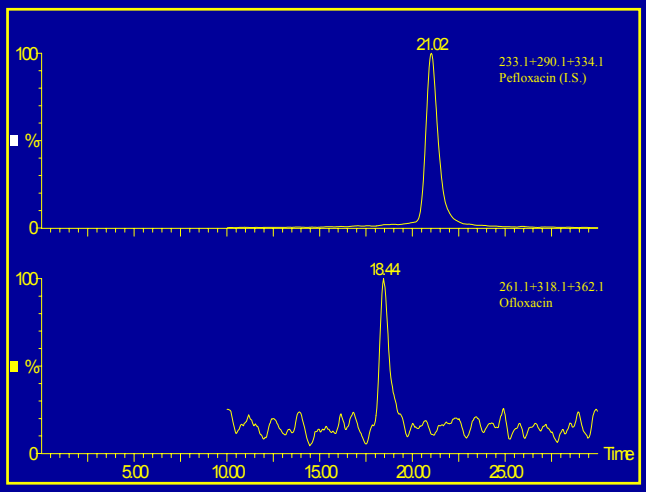

Fig.1: Reconstructed ion fragmentograms obtained for the clinical sample

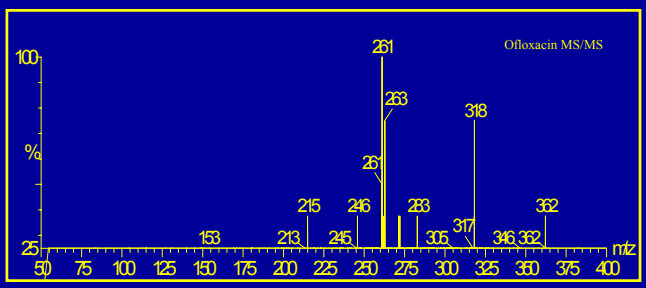

Fig.2: Diagnostic product ion spectrum of ofloxacin in the clinical sample

5. Conclusion

We clearly showed that the precipitate, removed by corneal scraping from the 6-year old boy with VKC, contained at least 125 pg of ofloxacin. Since treatment had been discontinued for seven days and in view of the short half-life of ofloxacin, our findings indicate that, at least in the treatment of VKC associated ulcers, deposits can occur after the topical use of ofloxacin.

6. Acknowledgements

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