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Discriminating chloramphenicol isomers by LC-MS/MS

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1. Introduction

Chloramphenicol (CAP) is a banned broad-spectrum antibiotic with historical veterinary use in all major food-producing animals. In total eight isomeric configurations of CAP exists (Figure 1) of which only RR-p-CAP has antimicrobial properties. To confirm the identity of any of the CAP isomers in suspect samples a highly specific method is needed. For many compounds the use of liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) is sufficiently specific because compound specific precursor and product ions are monitored. However, the existence of isomers, which all produce the same precursor ion, is a complicating factor. The possibility to discriminate all eight CAP isomers using electrospray (ESI) LC-MS/MS is presented here.

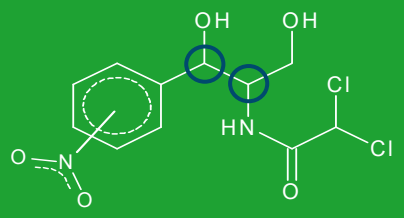


Figure 1. Chloramphenicol occurs in the para- and meta-configuration and it contains two chiral centers indicated by blue circles.

2. Mass spectrometry (ESI negative)

- Product ions: triple quadrupole MS.
- Product ion molecular formulas: Quadrupole-Time of Flight MS exact mass data.
- Fragmentation pathways: ion trap MS^n.

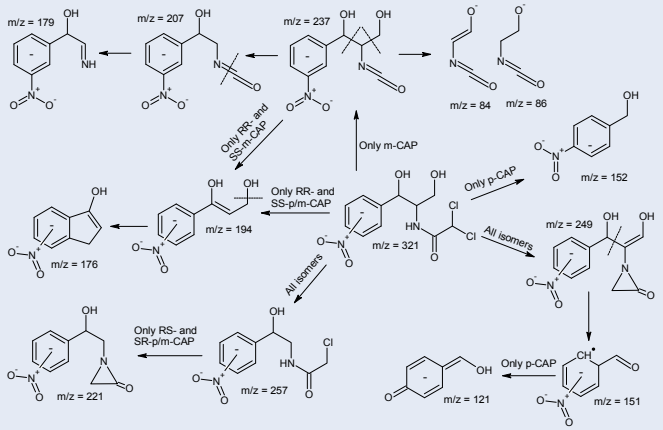


Figure 2. Product ions and fragmentation pathways of the CAP isomers

3. Chromatography

- High resolution reversed phase (RP) LC: 2.1×100 mm, 1.7 µm Acquity UPLC BEH C_{18} analytical column; methanolic gradient; 0.4 ml min^{-1}.
- Chiral LC: 2.0×150 mm, 5 µm α1-acid glycoprotein analytical column; a slight methanol/acetonitrile (1:1 v/v) two step gradient; 0.5 ml min^{-1}.

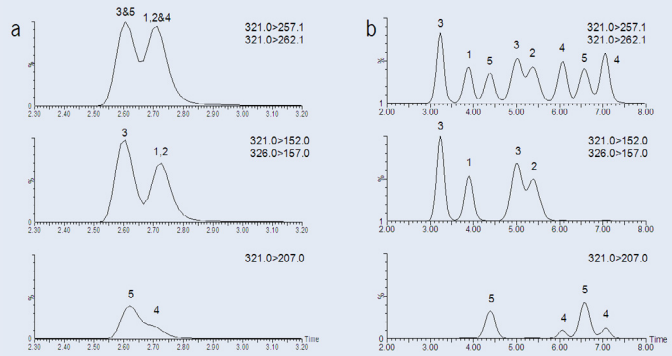


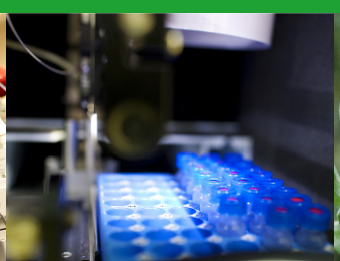
Figure 3. (a) High resolution RP-LC and (b) chiral LC chromatograms showing three MS transitions of a mixture of all CAP isomers. 1=RR-p-CAP, 2=SS-p-CAP, 3=RS- or SR-p-CAP-d_5, 4=RR- or SS-m-CAP and 5=RS- or SR-m-CAP.

4. Discussion

- Different product ions are obtained for the meta- and para-isomers as well as for RR-/SS- and RS-/SR-CAP. The highly abundant product ion m/z=152 is only observed for para-CAP and is absent for the meta-isomers. This is most likely caused by mesomeric stabilization of the benzylic anion which only occurs if the nitro group is in the para position. In the RR- and SS-isomers the amide nitrogen is located close to the hydroxyl and hydroxymethyl group indicating that it could be part of an intra-molecular hydrogen bridging system, which might cause different fragmentation compared to RS-/SR-CAP.
- Using RP chromatography the RS- and SR- isomers are separated from the SS- and RR-isomers. Although the applied system was not specifically optimized to achieve stereoisomeric separation, based on the exact co-elution of the stereoisomers it is unlikely that full separation of all isomers is achievable. Therefore it is concluded that, even in combination with MS detection, RP-LC-MS/MS is not able to discriminate all CAP isomers. Using chiral LC all CAP isomers can be (almost base line) separated.

5. Conclusion

- The pure form of all isomers, except for the mirror images (eg. RR-p- and SS-p-CAP), can be distinguished by MS/MS detection only if the product ions monitored are carefully selected.
- A chiral LC-MS/MS system is suited for isomeric specific confirmatory analysis of CAP.

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