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A new approach to analysing ceftiofur and cephapirin residues including metabolites in kidney

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1. Introduction

A high number of ß-lactam suspect samples were found in routine samples by our microbiological department and only a small number of these samples were identified as penicillin positive indicating that a reasonable number could contain cephalosporin antibiotics. Ceftiofur and cephapirin are instable cephalosporins and rapidly metabolize after intramuscular administration and it is not clear that currently applied methods take all relevant metabolites and degradation products into account. If not it is possible that total ceftiofur and cephapirin residue levels are underestimated.

Within our research, state-of-the-art techniques were used to identify ceftiofur and cephapirin degradation products and based on the results a new approach was developed for analysing ceftiofur and cephapirin residues including metabolites in kidney.

2. Experimental

The degradation of ceftiofur, desfuroylceftiofur (DFC), cephapirin and desacetylcephapirin (DAC) was tested (1) at elevated temperatures, (2) in the presence of kidney extract and (3) in alkaline solution. For each condition blank and spiked samples were prepared and incubated (37°C) for 0 through 72 hours. The resulting products were detected using LC-ToF/MS by subtracting chromatograms of blank samples from spiked samples (MetAlign software). A suggestion for the molecular structure of the degradation products was made based on a combination of LC-ToF/MS (exact mass and isotope ratio), LC-QqQ/MS (fragmentation), NRM (structural information) and microbial techniques (microbial activity). The degradation kinetics were determined using LC-ToF/MS.

3. Results & discussion

After addition of ceftiofur and cephapirin to kidney extract complete degradation occurred instantaneously resulting in both formerly reported and unknown degradation products. Some interesting products are presented in figure 1.

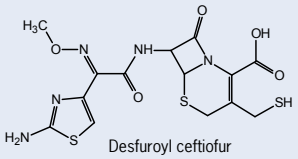
4. Conclusion

It is concluded that conditions often occurring during the analysis of ceftiofur or cephapirin can result in rapid degradation to known and newly identified products. Consequently it is suggested that by using conventional measurement approaches an underestimation of the determined amount of cephalosporins can occur.

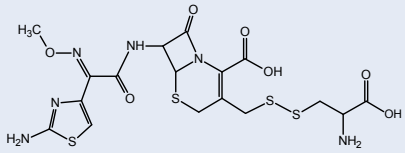
5. A new approach

Many different degradation products for ceftiofur were detected in kidney extract but after addition of ammonia, during incubation at 37°C, ATMA is produced which remained stable (fig 2). Under the same conditions from cephapirin the analogous PTA is produced[†].
A new approach is suggested in which ATMA is selected as a marker compound of ceftiofur and PTA of cephapirin. A method was developed for forced degradation of ceftiofur and cephapirin to ATMA and PTA followed by a clean-up using Solid Phase Extraction and detection using LC-QqQ/MS. This method proved suitable for quantification of ceftiofur and cephapirin in spiked kidney samples at MRL level. When incurred samples are available this approach has to be tested and compared with currently applied methods.

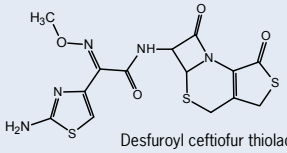
In the presence of kidney extract:



Desfuroyl ceftiofur

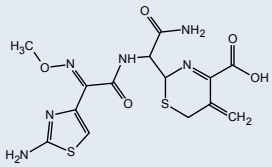


Desfuroyl ceftiofur cysteine disulfide

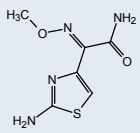


Desfuroyl ceftiofur thiolactone

After addition of ammonia:



Ammoniated desthiofuroyl ceftiofur



ATMA

Figure 1. Detected degradation products for ceftiofur

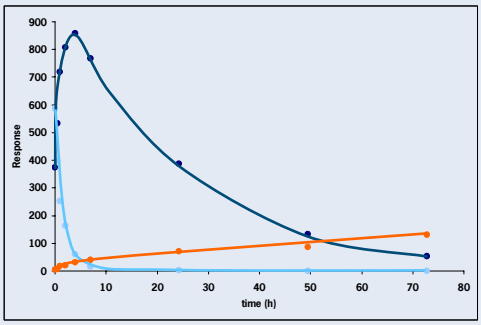


Figure 2. The kinetics of (●) DFC cysteine disulfide, (●) Ammoniated desthiofuroylceftiofur and (●) ATMA produced from ceftiofur in alkaline kidney extract during incubation at 37°C. No ceftiofur was detected.

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[†] Details on ceftiofur and cephapirin were published in: B.J.A. Berendsen, Martien L. Essers, Patrick P.J. Mulder, Gerard D. van Bruchem, Arjen Lommen, Wendy M. van Overbeek and Linda (A.)A.M. Stolker. 2009. J. Chrom. A. 1216 (46).