A RAPID MULTIRESIDUE METHOD FOR THE DETERMINATION OF SULFONAMIDE AND ß-LACTAM RESIDUES IN BOVINE MILK

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Introduction
Various sulfonamide and ß-lactam antibacterial compounds may be used to treat disease in lactating dairy cattle and it is therefore necessary to monitor milk for the presence of residues of these drugs. In the European Union, maximum residue level (MRL) values range from 100 ppb, as a total concentration of all sulfonamides, to 4 ppb for each of the penicillin compounds amoxillin and ampicillin. In the USA, the tolerance levels are 10 ppb for both these penicillin compounds, but the use of most sulfonamides is prohibited in cattle used for milk production.

Multiresidue analyses are increasingly gaining acceptance for the determination of residues in foodstuffs; methods have recently been published for the monitoring of over 150 pesticide compounds in fruit and vegetables. Various classes of pesticide compound may be detected, in a variety of produce types, using generic extraction and analytical methods. The sample preparation method must be non-selective in order to obtain acceptable recovery for all target residues. This results in a complex sample matrix that has the potential to interfere with the determination of the various analytes. To compensate for this, it is necessary to have a selective, but at the same time universal, determination of target residues. Waters tandem-quadrupole mass spectrometry (MS/MS) instruments provide the necessary selectivity to give low detection levels in the presence of co-extractives, while simultaneously providing universal detection of all analytes.

Multiresidue MS/MS techniques are increasingly being applied to the monitoring of veterinary drug residues in food of animal origin. Most methods published to date target a relatively small number of analytes from a particular class of compounds. This work is intended as an initial step in the development of a multi-class, multiresidue method for veterinary drugs. It describes a method for the analysis of 15 sulfonamide compounds, together with a number of penicillins and cephalosporins, in bovine milk.

Method
Three recovery samples at 4 ppb and three at 40 ppb were prepared. Matrix matched external calibration standards were prepared at 0, 1, 10, 20, 50 and 100 ppb. Since 1 mL milk is equivalent to 0.5 mL final extract, a concentration of 1 ppb residue in milk is equivalent to a concentration of 2 ng/mL in the final extract.

Extraction
1 mL aliquots of pasteurized, homogenized cows milk, containing 4% fat, were transferred to 2 mL polypropylene sample tubes. Recovery samples were spiked, agitated and left to equilibrate for 30 minutes. In order to separate the lipid from the aqueous portion, the tubes were centrifuged at 13,000 rpm for 10 minutes. The aqueous layers were transferred to Oasis HLB solid phase extraction (SPE) columns containing 60 mg of material. The Oasis HLB columns had previously been conditioned with 1 mL methanol and 1 mL water. The polypropylene tubes were washed with 2 × 1 mL aliquots of water, which were added to the Oasis SPE columns. Samples were drawn through under vacuum and the column was washed with 1 mL water. Analytes were eluted in 1 mL methanol. The methanol eluent was evaporated to near dryness at 50 °C under vacuum, and the samples reconstituted in enough water to give a final volume of 0.5 mL. Calibration standards were spiked at this point.

Chromatography
Chromatographic separation was carried out using a Waters Alliance 2795 HPLC System. The LC column was a Waters 4.6 mm id by 150 mm X Terra RP18 with 3.5 µ particle size.
Mobile phase A  20 mM ammonium formate in water, pH adjusted to 3.5 with formic acid

Mobile phase B  20 mM ammonium formate in 90% methanol, pH adjusted to 3.5 with formic acid

Injection volume  20 μL
Flow rate  0.6 mL/min

Gradient program is shown in Table 1.

<table>
<thead>
<tr>
<th>Time/min</th>
<th>0 min</th>
<th>0.5 min</th>
<th>10 min</th>
<th>12 min</th>
<th>12.1 min</th>
<th>16 min</th>
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<td>% A</td>
<td>100 A</td>
<td>100 A</td>
<td>0 A</td>
<td>0 A</td>
<td>100 A</td>
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</table>

Table 1. Chromatographic gradient.

Mass Spectrometry

The eluent from the LC column was directed into the electrospray source of a Waters Micromass® Quattro Premier™ tandem quadrupole mass spectrometer operated in positive ionisation mode. Two multiple reaction monitoring (MRM) transitions were monitored for each compound. Table 2 gives details of the source cone and collision cell voltages for each transition.

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time/min</th>
<th>Cone Voltage/V</th>
<th>Parent ion m/z</th>
<th>Daughter ion 1 m/z</th>
<th>Daughter ion 2 m/z</th>
<th>Collison Voltage 1/V</th>
<th>Collison Voltage 2/V</th>
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<td>215</td>
<td>156</td>
<td>15</td>
<td>92</td>
<td>26</td>
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<td>424</td>
<td>292.2</td>
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Table 2. MRM parameters.
Figure 2. Chromatograms from the two MRM transitions monitored for amoxicillin.

Figure 3. Calibration graph for amoxicillin.

Figure 4. Chromatograms from the two MRM transitions monitored for cephapirin.

Figure 5. Calibration graph for cephapirin.

Figure 6. Chromatograms from the two MRM transitions monitored for sulfathiazole.

Figure 7. Calibration graph for sulfathiazole.
Figure 8. Chromatograms from the two MRM transitions monitored for sulfaguanidine.

Figure 9. Calibration graph for sulfaguanadine.

Figure 10 contains a graph showing the mean percentage recovery at 40 ppb. These range between 75% and 112%.

Figure 10. Efficiency of solid phase extraction method for all analytes.
Figure 11 shows an estimate of limit of detection. These values were calculated from a 4 ppb recovery standard and are an estimate of the concentrations that would be expected to give a signal to noise value of 3:1, using the most abundant MRM transition.
Conclusion

Using a Waters chemistry solution, a simple and generic solid-phase sample extraction method was applied to 21 veterinary drug residues in bovine milk. When the samples were analysed using a Waters LC/MS/MS system the method was able to quantify and confirm the presence of these residues well below the required maximum residue limits/tolerance levels set by both the European Union and US FDA. The method may be extended to include other residues and residue classes.

References

1. European Union Council Regulation No. 2377/90
2. United States Food and Drug Administration, Code of Federal Regulations, Title 21, Parts 530 and 556
4. G. Kearney, L. Alder and A. Newton, The Advantages of Multiple Reaction Monitoring over Single Ion Recording for the Analysis of 81 Pesticide Residues in Fruit and Vegetables, Waters Application Note 720000693EN
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