Waters™

응용 자료

A Rapid Multi-Residue Method for the Determination of Sulfonamide And β-Lactam Residues in Bovine Milk

Gordon Kearney

Waters Corporation

Abstract

The work carried out in this application note 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.

Introduction

Various sulfonamide and β-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.[1] 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.[2]

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.[3] 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.[4]

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.[5,6] 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.

Experimental

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 XTerra RP_{18}, 4.6 mm x 50 mm, 3.5 µm.

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.

Time/min	0 min	0.5 min	10 min	12 min	12.1 min	16 min
	100% A	100% A	0% A	0% A	100% A	100% A

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.

Name	Retention Time/min	Cone Voltage/V	Parent ion/ m/z	Daughter ion 1/m/z	Collision Voltage 1/V	Daughter ion 2/ m/z	Collision Voltage 2/V
Sulfaguanidine	5.71	30	215	156	15	92	26
Sulfanilimide	5.91	10	173	156	7	92	17
Amoxicillin	6.74	18	366	114	20	208	13
Cephapirin	7.40	32	424	292.2	16	152	24
Sulfadiazine	7.53	30	251	156	16	92	26
Sulfathiazole	7.70	28	256	156	15	92	28
Sulfapyridine	7.93	30	250	156	16	92	27
Sulfamerazine	8.12	32	265	156	18	92	28
Sulfameter	8.51	30	281	156	18	92	30
Sulfamethizole	8.55	26	271	156	14	92	28
Cefazolin	8.55	18	455	323	11	156	15
Sulfamethazine	8.66	30	279	186	17	92	30
Sulfamethoxypyradizine	8.76	32	281	156	16	92	30
Cefoperazone	8.78	22	645.9	530	12	143	35
Ampicillin	8.9	25	350	160	12	106	17
Sulfachloropyridazine	9.08	28	285	156	15	92	28
Sulfamethoxyazole	9.12	28	254	156	16	92	26
Sulfamonomethoxine	9.34	32	281	156	18	92	30
Sulfadimethoxine	10.19	40	311	156	20	92	32
Sulfaquinoxaline	10.41	35	301	156	18	92	30
Penicillin G	11.33	45	335	128	27	91	45

Table 2. MRM parameters.

Results and Discussion

Figure 1 shows the total ion current (TIC) chromatogram obtained from the analysis of a 40 ppb recovery standard. All peaks elute between 5.5 and 11 minutes.

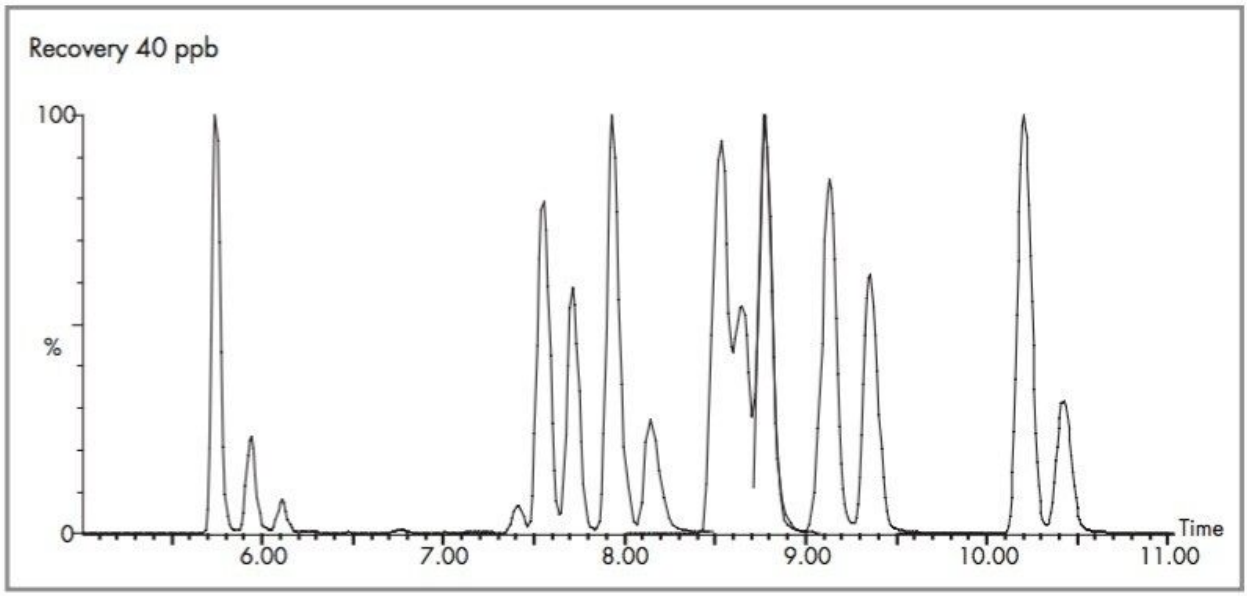

Figure 1. TIC chromatogram for all analytes.

Figures 2 to 9 show example chromatograms and calibration graphs for amoxicillin, cephapirin, sulfathiazole, and sulfaguanidine.

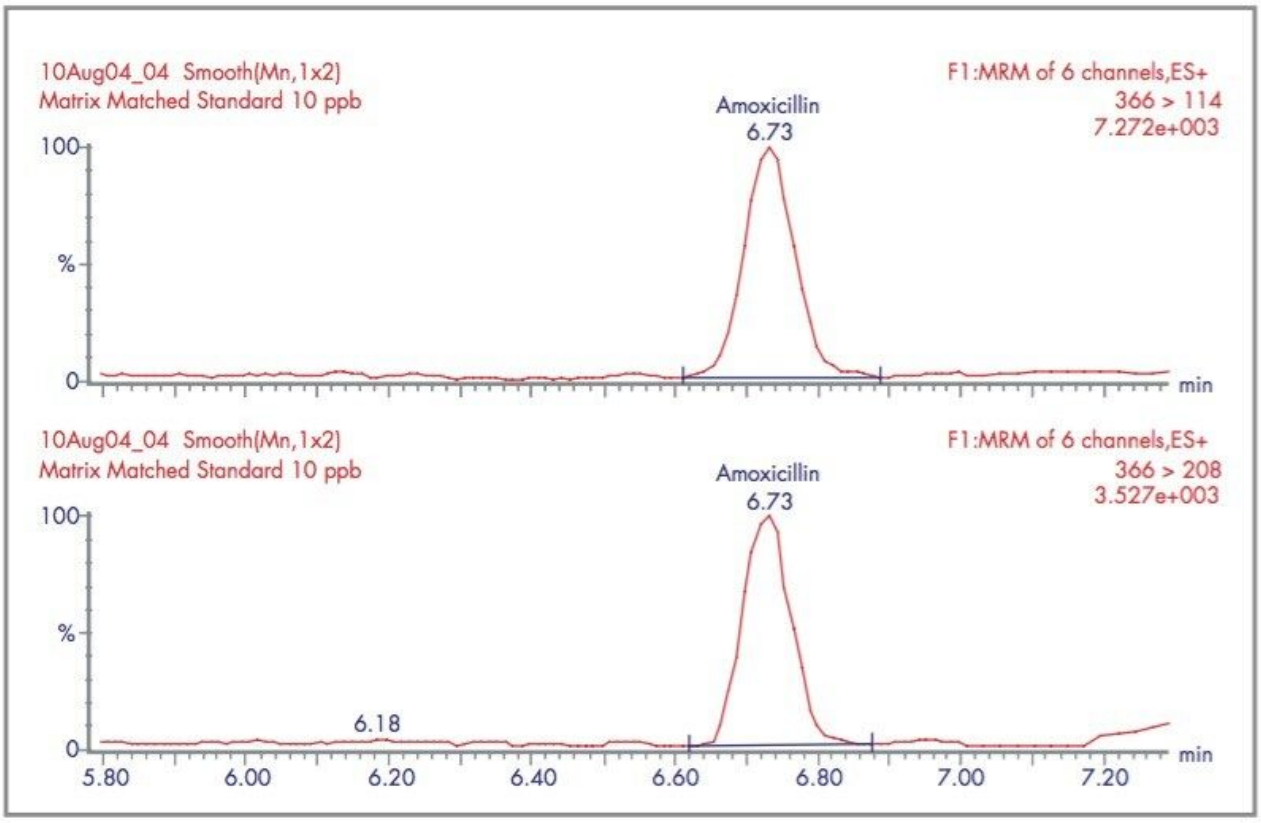

Figure 2. Chromatograms from the two MRM transitions monitored for amoxicillin.

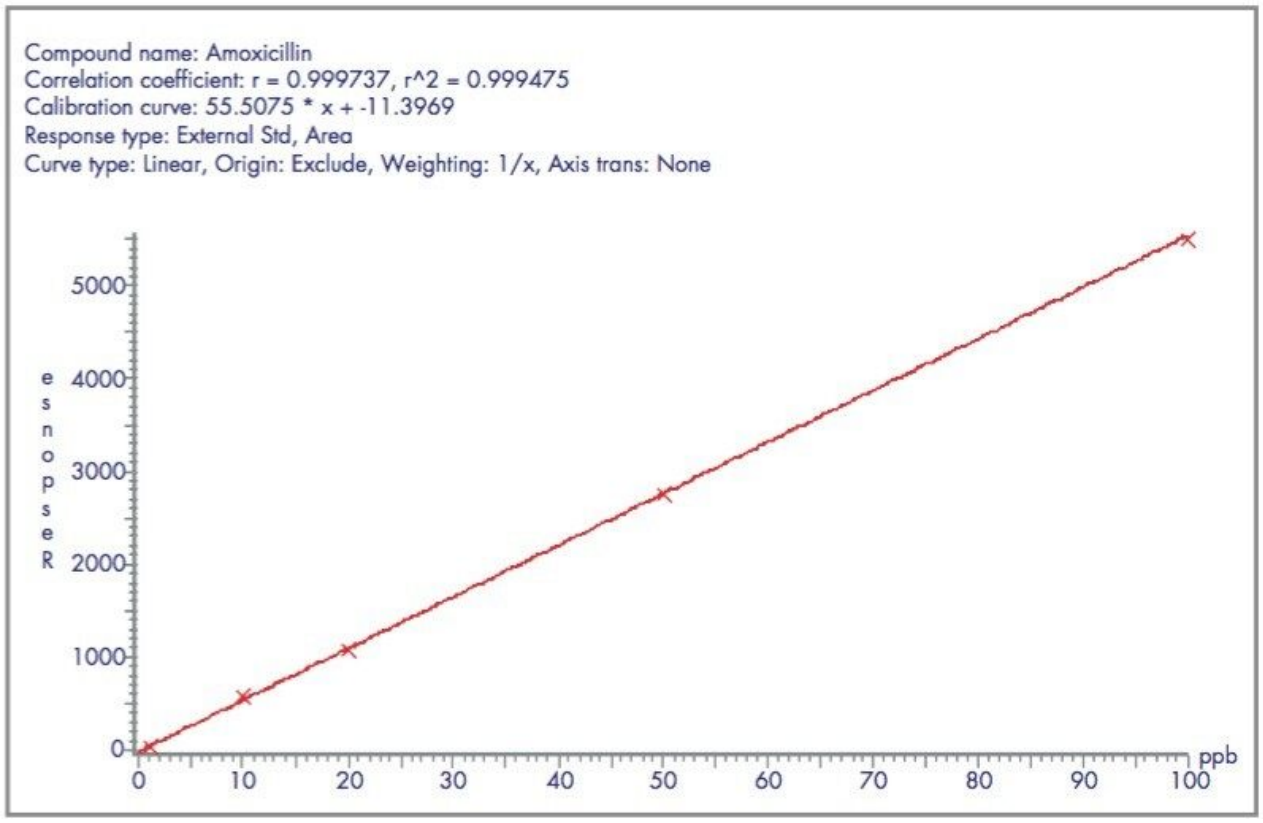

Figure 3. Calibration graph for amoxicillin.

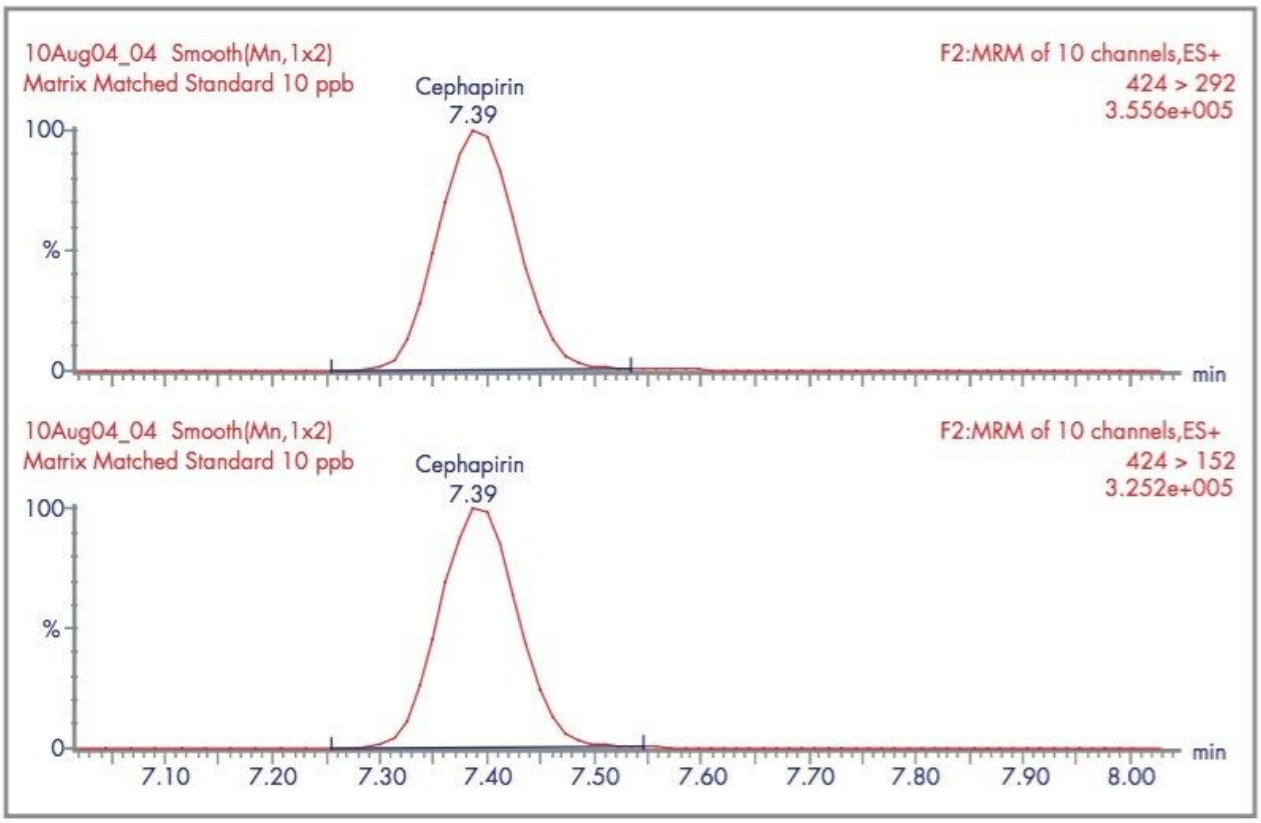

Figure 4. Chromatograms from the two MRM transitions monitored for cephapirin.

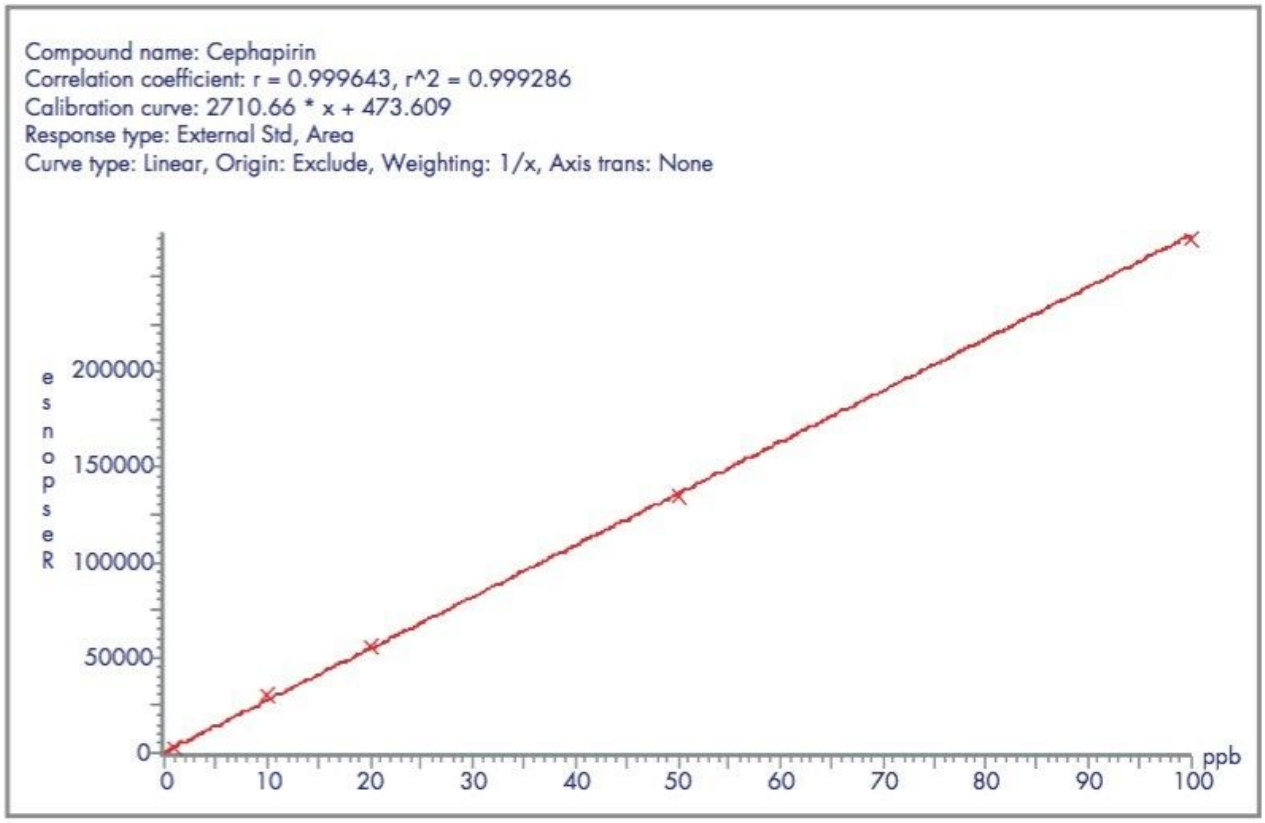

Figure 5. Calibration graph for cephapirin.

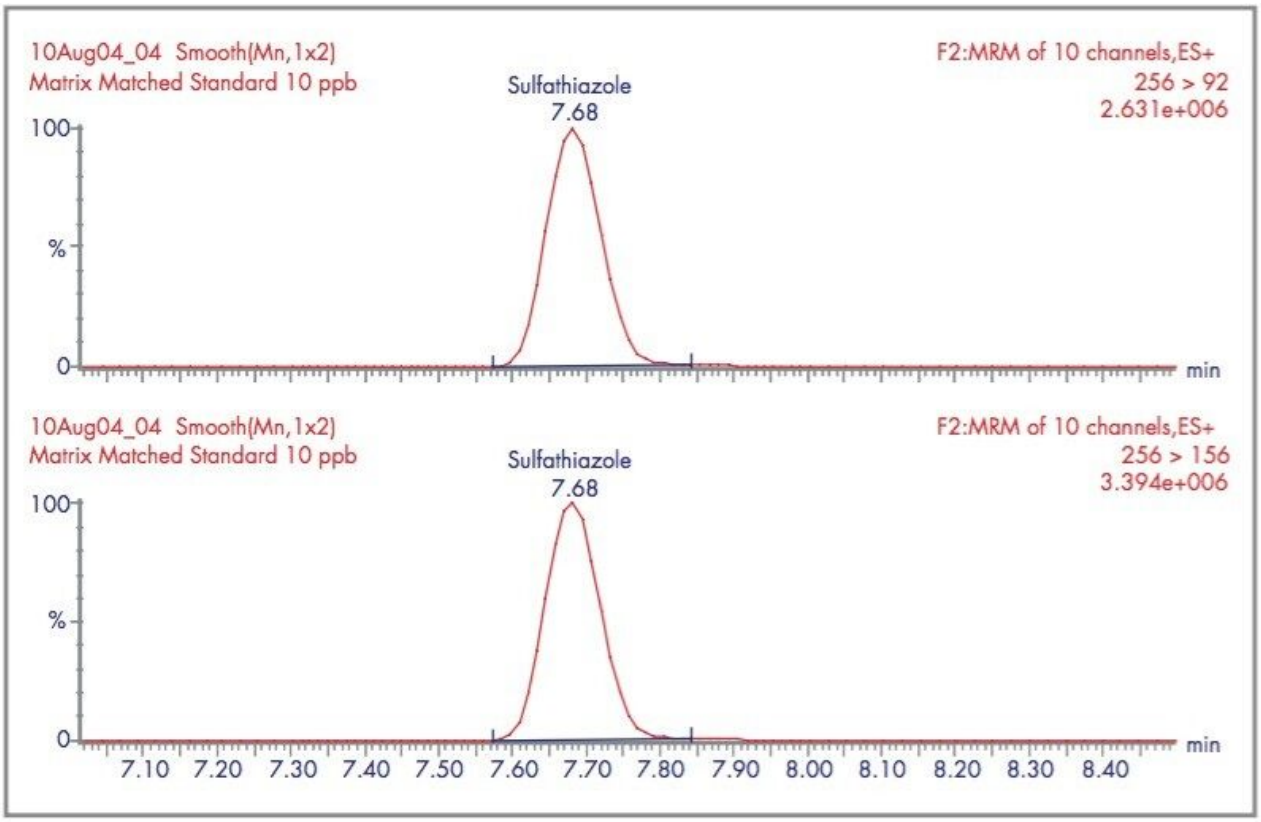

Figure 6. Chromatograms from the two MRM transitions monitored for sulfathiazole.

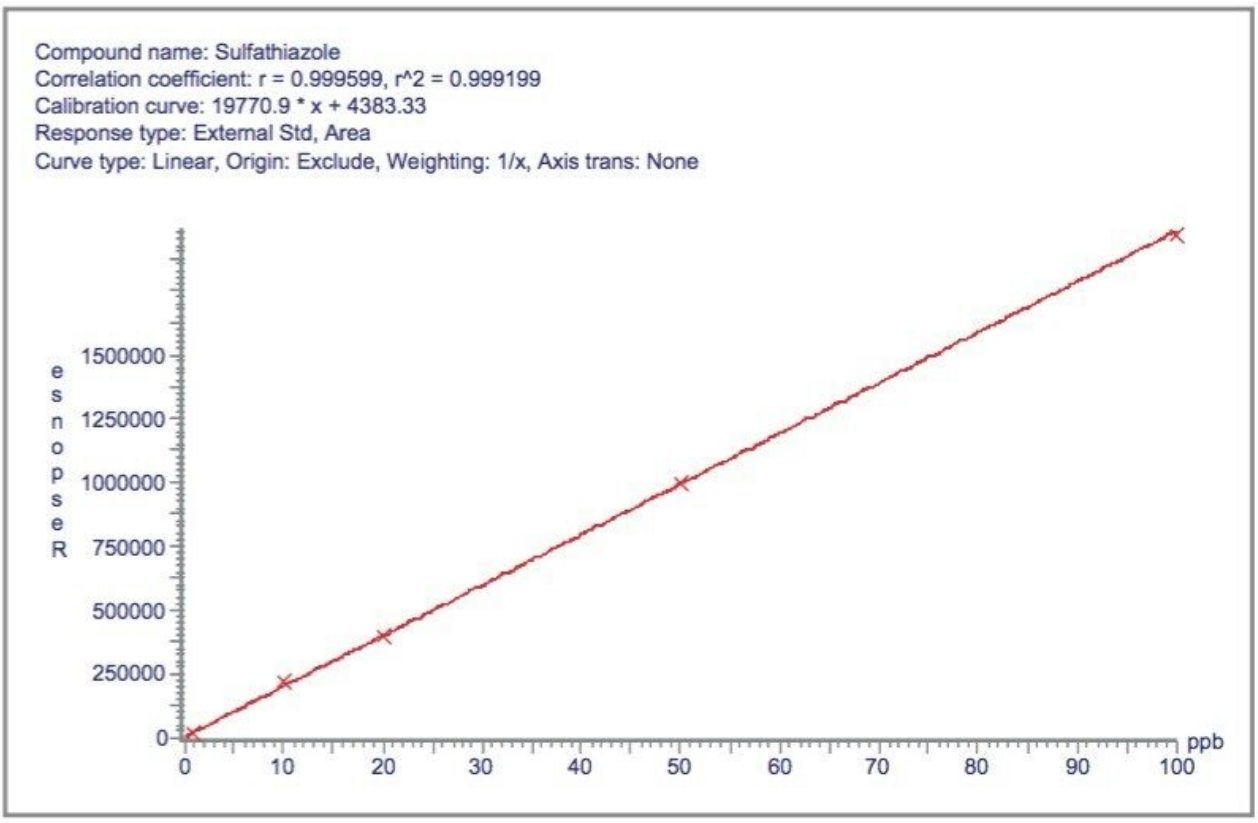

Figure 7. Calibration graph for sulfathiazole.

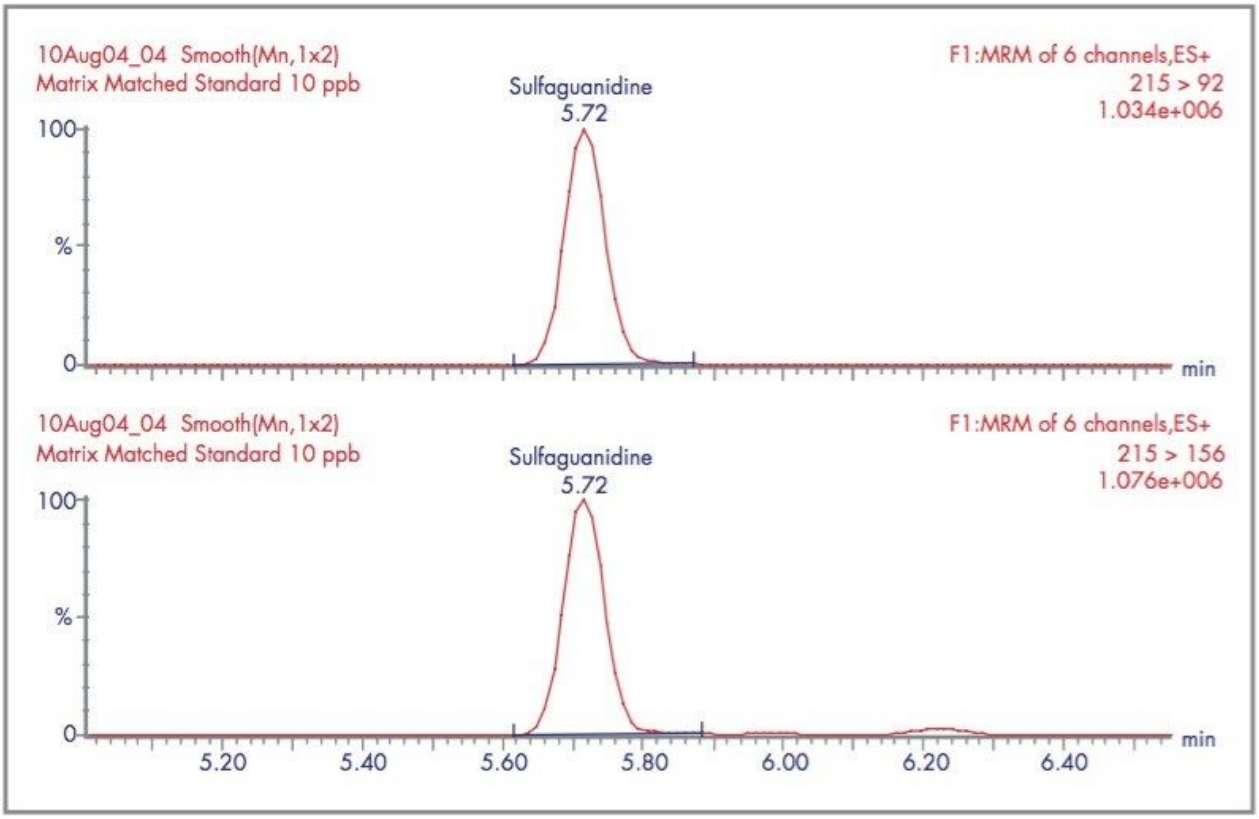

Figure 8. Chromatograms from the two MRM transitions monitored for sulfaguanidine.

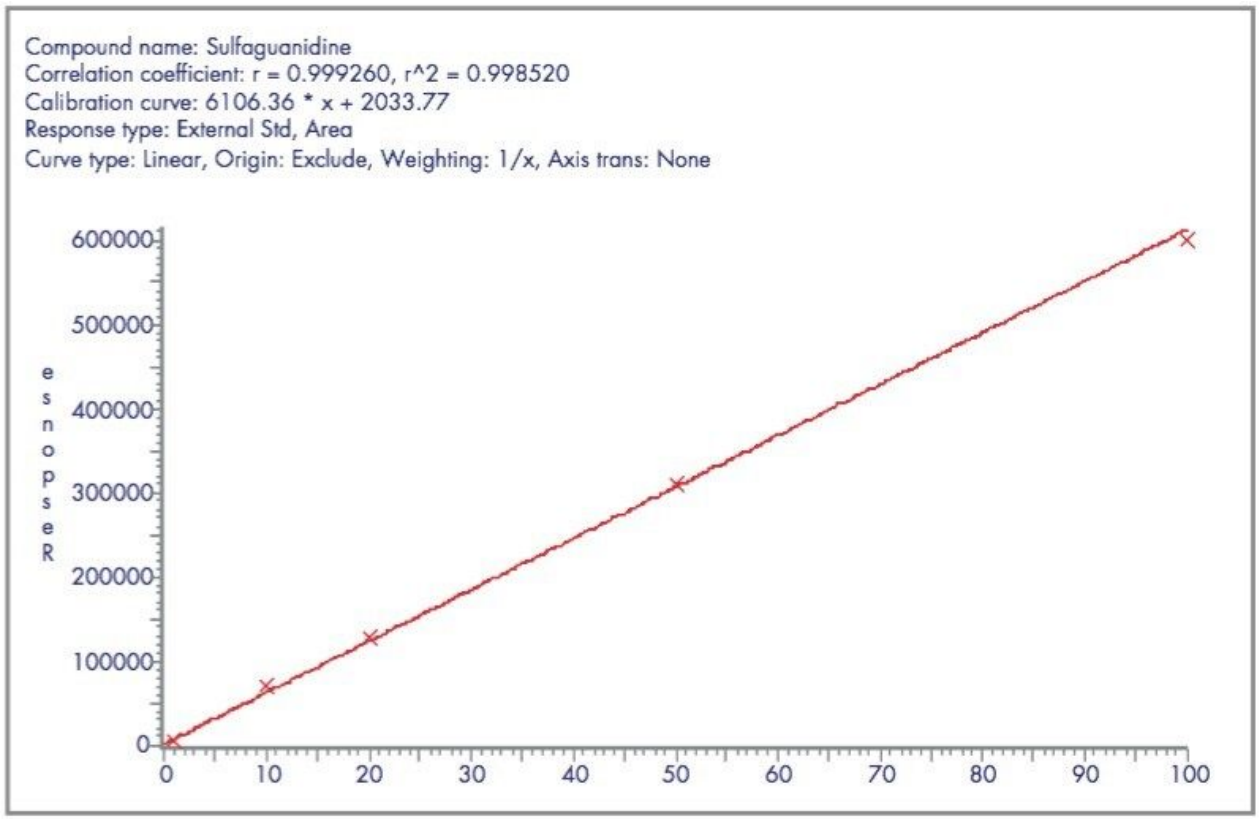

Figure 9. Calibration graph for sulfaguanadine.

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

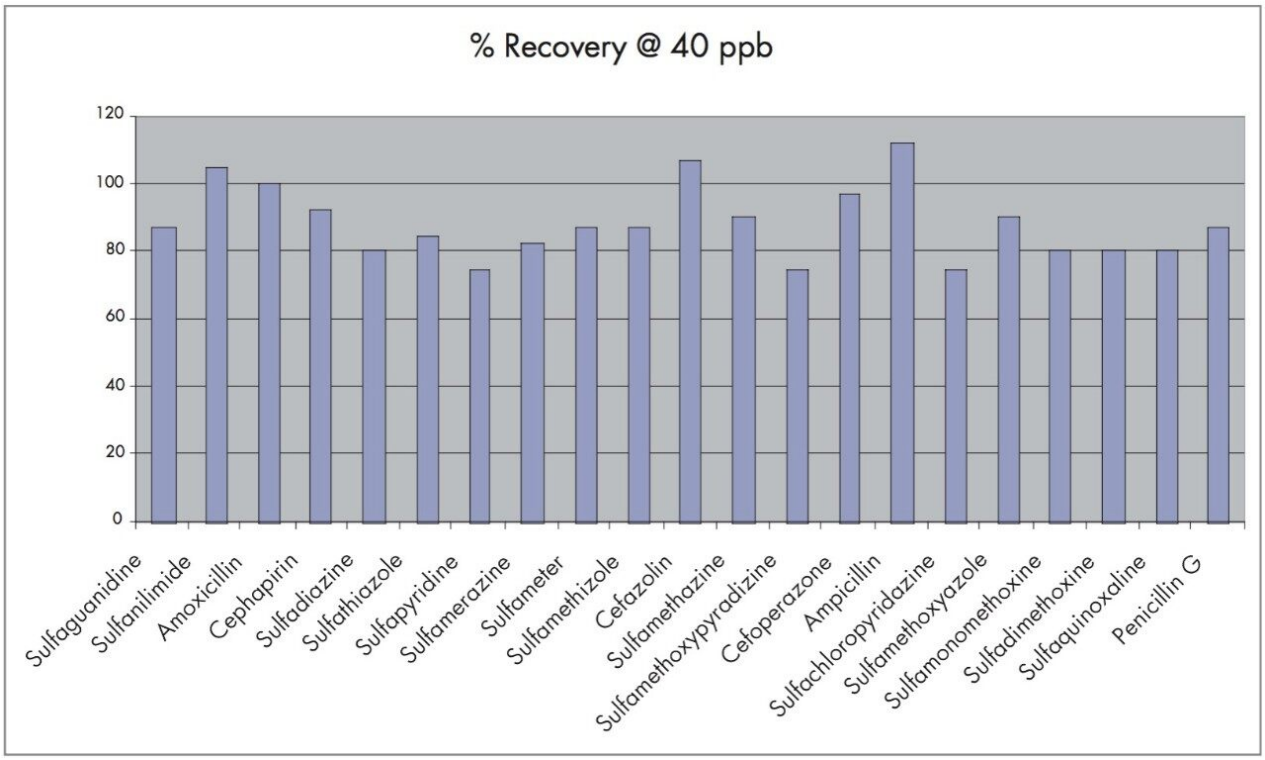

Figure 10. Efficiency of solid phase extraction method for all analytes.

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.

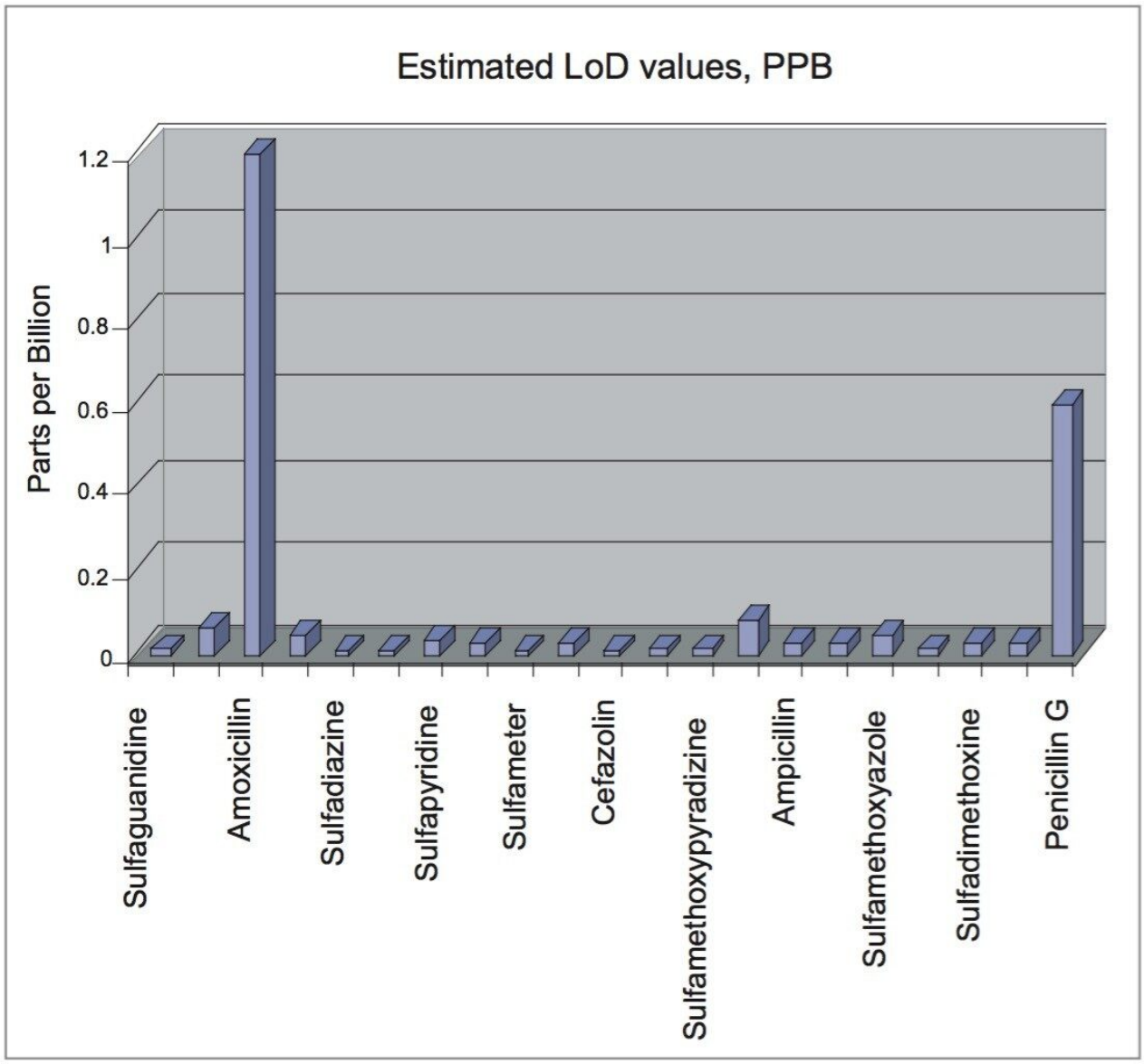

Figure 11. Estimated limit of detection for all analytes.

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
3. New Strategies for Comprehensive Multiresidue Analysis Using GC and LC with Various MS and MS/MS Detection Techniques, André de Kok, VWA-Food and Consumer Product Safety Authority, The Netherlands. Presented at the 5th European Pesticide Residue Workshop, June 13th to 16th, 2004, Stockholm, Sweden.
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 <https://www.waters.com/nextgen/us/en/library/application-notes/2004/%20the-advantages-of-multiple-reaction-monitoring-mrm-over-single-ion-recording-sir-for-the-analysis-of-81-pesticide-residues-in-fruit-and-vegetables.html>
5. C. Cavaliere, R. Curini, A. Di Corcia, M. Nazzari and R. Samperi, *J. Agric. Food Chem.*, 2003, 51, pp 558–566
6. S. Riediker, J. Diserens, R. Stadler, *J. Agric. Food Chem*., 2001, 49, pp 4171–4176

Featured Products

Alliance HPLC System <https://www.waters.com/534293>

720001065, December 2004