

**METHODS FOR VETERINARY DRUG RESIDUE  
ANALYSIS IN FOOD**

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# **METHODS FOR VETERINARY DRUG RESIDUE ANALYSIS IN FOOD**

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## SUMMARY

A comprehensive capability to test for residues of veterinary drugs is an important support for the Irish food industry. There is a requirement for food manufacturers to demonstrate the compliance of their products with stringent customer specifications. In addition, legislative requirements are becoming more exacting both in terms of the range of substances covered and the lower residue levels to which test systems must measure.

The objective of this project was to address a number of analytical problems associated with veterinary drug residues particularly in the areas of growth promoting agents and antimicrobial substances. Specific needs for multi-residue methods for  $\beta$ -agonists and antimicrobial substances and for analysis of the growth promoter, zeranol, were addressed.

- Methods for  $\beta$ -agonists have been developed, validated and applied to test samples. The basis for these methods is the use of sorbents (chromatographic materials) in new formats to extract the residues from tissues and body fluids. A matrix solid phase dispersion (MSPD) method coupled with solid phase extraction (SPE) clean-up and determination by immunoassay gave high recovery (>80%) for a range of  $\beta$ -agonist residues in liver and this method has a sensitivity of less than 1 ppb clenbuterol. Other methods were developed using mixtures of sorbents, in SPE and MSPD modes, to analyse  $\beta$ -agonists in urine and liver samples. The particular advantage of these methods is that, by use of a combination of the chemical properties of different sorbents, a complex extraction and clean-up procedure can be achieved within a single, relatively-straightforward procedure.
- Chromatographic separation coupled with immunoassay determination, the “immunogram” approach, has been developed to study the metabolite profile obtained in bovine urine samples following either treatment with the prohibited growth promoter zeranol (Ralgro®) or feeding with the mycotoxin zearalenone (a potential natural contaminant of animal feed). This method is capable of distinguishing samples from animals treated with the prohibited growth promoter from samples contaminated due to ingestion of mycotoxin from contaminated feed.
- Multi-residue methods have been developed for sulphonamides using the technique of automated dialysis, for  $\beta$ -lactams (penicillins) using MSPD and for tetracyclines using LC-MS. The methods allow food products such as pork, to be tested for a range of antimicrobial residues. The sensitivities for these methods are sufficient to allow for testing at levels below the appropriate maximum residue limits (MRLs) for the antimicrobials (50 to 300 ppb).

## **ANALYTICAL METHODS FOR $\beta$ -AGONISTS**

The  $\beta$ -agonists, or partitioning agents, have been used very widely throughout Europe as illegal growth promoters in beef and veal production, following the ban on anabolic agents in the mid-1980s. Serious incidences of poisoning occurred due to very high levels of the  $\beta$ -agonist clenbuterol in beef and veal liver in Spain and France, respectively (Martinez-Navarro, 1990; Pulce et al., 1991). A study carried out by the European Consumers Associations in 1994 on retail-purchased liver in all countries within the EU found residue-positive samples at high numbers in some countries: Spain 36%, Belgium 23%, France 13%, The Netherlands 10% (Remy and Debeuckelaere, 1994).

In Ireland, illicit use of  $\beta$ -agonists in beef production was suspected in the late 1980s and early 1990s. Concerted actions by the Department of Agriculture and Food in the early 1990s and severe penalties on producers using prohibited growth promoters appear to have reduced the illicit use of these agents.

Because of the importance of the quality image of Irish beef and the need for the best analytical capability at The National Food Centre to support the Irish beef industry, projects were undertaken to develop efficient and sensitive methods for  $\beta$ -agonist residues determination in tissues and body fluids. The development of sorbent technologies, including solid phase extraction (SPE) and matrix solid phase dispersion (MSPD), for residue extraction, and immunoassays and mass spectrometry for residue determination offered the best approach to methods for  $\beta$ -agonists.

### **Matrix solid phase dispersion method for $\beta$ -agonists in bovine liver**

Liver, because it is the edible tissue in which  $\beta$ -agonist residues persist for longest, is the most suitable matrix for analysis to establish that beef carcasses do not contain measurable residues. While clenbuterol has been the most widely used  $\beta$ -agonist for growth promotion, a multi-residue procedure suitable for other  $\beta$ -agonists, such as salbutamol and mabuterol, was required to monitor for illicit use of the range of substances.

Earlier work on MSPD had produced methods for clenbuterol (Boyd et al., 1993) and for  $\beta$ -agonists (Boyd et al., 1994). The performance of this latter method, however, was found to be highly dependent on the type of sorbent used so a procedure involving coupling of MSPD with SPE was developed, as shown in Figure 1 (Boyd et al., 1995).

The performance of this method was assessed through “recovery studies”, i.e. addition of a known quantity of the individual  $\beta$ -agonists to a residue-free liver sample and determination of the amount of the added residue recovered by the method. The results achieved for clenbuterol, salbutamol and mabuterol are shown in Figure 2; mean values for recovery of the three  $\beta$ -agonists added at concentrations of 1 and 2 ng/g (ppb) are shown together with the coefficient of variation (CV) of the mean, which gives a measure of the reproducibility of the method from one assay to another.

The mean recoveries of 80% (or higher) indicate that the method performs well and the CV values of between 12 and 26% indicate acceptable variation for a method measuring at 1 - 2 ppb and using immunoassay.

The method was further assessed for its performance on “real” samples, i.e. samples of liver obtained from animals which had been treated experimentally with clenbuterol and salbutamol. Figure 3 shows a comparison between the values obtained by this method and values obtained for the same samples by alternative methods; acceptable agreement between the different methods is obtained. In this figure, also, the results for analyses using C<sub>18</sub> sorbents from two manufacturers for the MSPD step indicate that the method is “robust” and is not strongly influenced by such variations. Such robustness is important in that it suggests that the method is likely to perform well in different laboratories under different conditions.

### **Mixed mode solid phase extraction method for $\beta$ -agonists in urine and liver**

Apart from edible tissues, such as liver, analyses for prohibited substances are often undertaken on body fluid samples, such as urine, bile or blood. Analysis of urine samples for  $\beta$ -agonists provides a suitable screening procedure for their use and may be undertaken on samples obtained from the living animal or post-slaughter.

Typically, SPE columns are used to extract the analyte(s) of interest from the urine sample and provide a purified extract for residue determination. Because of the very different chemistry of the  $\beta$ -agonists, a simple SPE step, using sorbent of one type, was found to be unsuitable. Mixed mode SPE columns, i.e. columns containing sorbent materials with different retention mechanisms, were applied to  $\beta$ -agonist extraction from urine and from primary liver extracts (Collins et al., 1994).

The procedure is shown in Figure 4 and the recovery of a range of  $\beta$ -agonists from liver and urine samples is shown in Figure 5. Mean recovery of  $\beta$ -agonists from urine and liver is >75% and >90%, respectively. The high recovery for the more polar  $\beta$ -agonists, salbutamol and terbutaline, as well as for the less polar clenbuterol and mabuterol is due to an efficient solvent extraction step from liver samples and the selective mixed mode solid phase extraction (SPE) provided by the XtrackT® columns. This mixed mode SPE procedure provides another efficient system for multi-residue  $\beta$ -agonist analysis in body fluid or primary tissue extract samples.

### **Mixed mode matrix solid phase dispersion method for $\beta$ -agonists in bovine liver**

While a single-step MSPD procedure for clenbuterol in liver was developed (Boyd et al., 1993) and a similar procedure for multi-residue  $\beta$ -agonists in liver had shown initial promise (Boyd et al., 1994), a robust procedure was not available (as discussed above) and multi-residue  $\beta$ -agonist analysis in liver required the coupling of two techniques, either MSPD with SPE or solvent extraction with SPE. The idea of incorporating the mixed mode approach into MSPD was assessed (Collins et al., 1996). In this procedure the liver sample was blended as normal with the C<sub>18</sub> sorbent

but the resulting blend was then mixed further with an ion exchange type sorbent, propylsulphonic acid (PRS) (Figure 6).

A series of experiments was undertaken, using radiolabelled clenbuterol and salbutamol, to establish the best type of reversed phase sorbent ( $C_{18}$  end-capped ( $C_{18}$  EC) or non end-capped ( $C_{18}$ )), the quantity of ion exchange (PRS) material required and the concentration of ammonia in methanol giving highest yield of the analytes. In summary, the best recovery of  $\beta$ -agonists was obtained when 1g of PRS was added to the tissue blended with end-capped  $C_{18}$  sorbent and when 8% ammonia in methanol was used for elution (Figure 7). As expected, salbutamol recovery was more affected by these variables than was the recovery of clenbuterol. Introduction of a further clean-up step with phosphate buffer (50 mm, pH 6.0), after the acetic acid activation of the PRS and prior to the methanol wash, provided a method which yielded recoveries of 86% and 70% for clenbuterol and salbutamol, respectively, from liver samples fortified at 2 ppb.

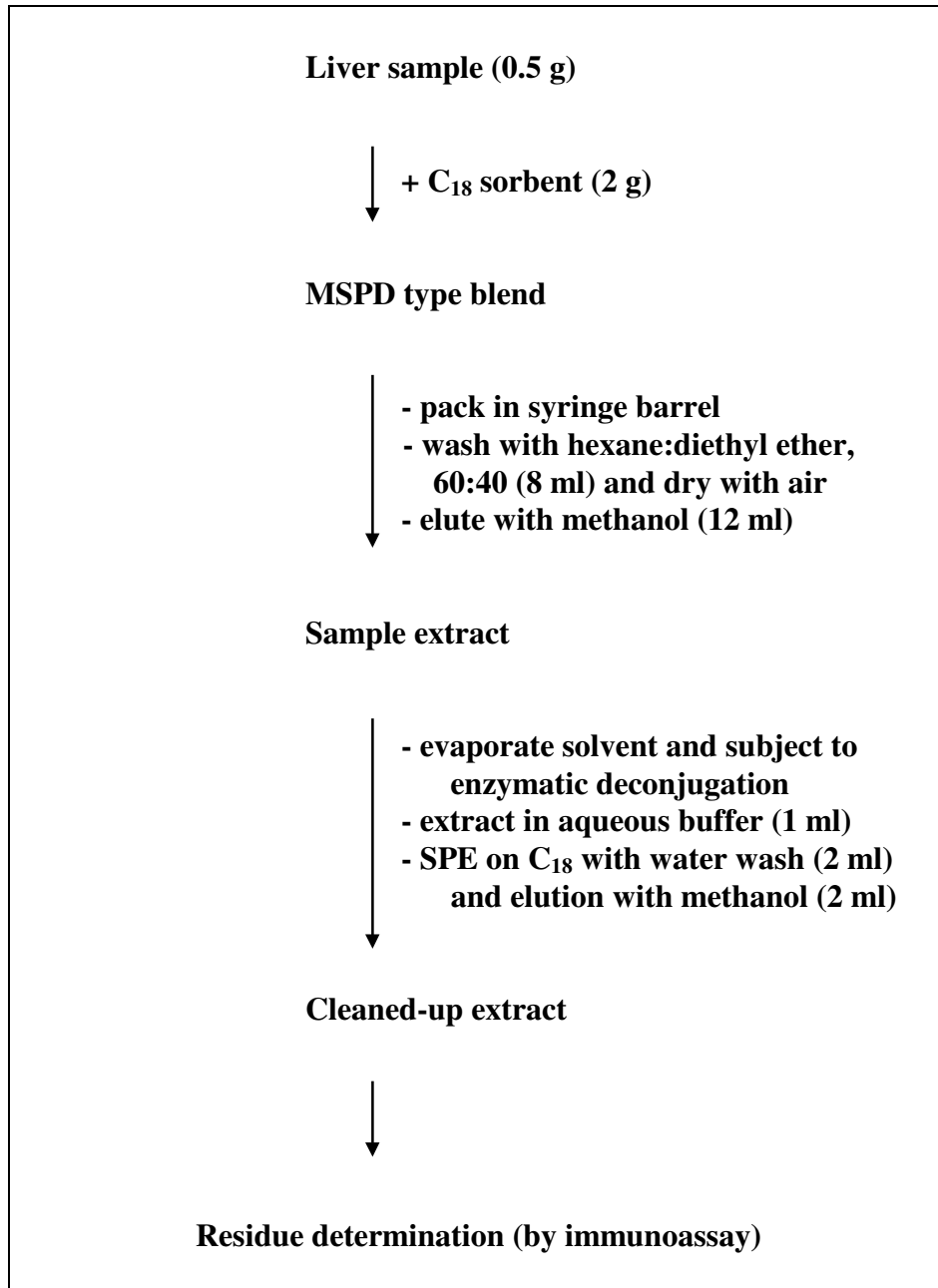
### **Gas chromatography - mass spectrometry analysis for $\beta$ -agonists**

While efficient methods for residue extraction coupled with immunoassay determination provide very useful methods for routine analysis for  $\beta$ -agonists, confirmatory procedures are required, also, to support the routine methods. Mass spectrometry (MS), because it gives structural information on the analyte, can supply unequivocal identification of the substance detected and, because MS can be interfaced with chromatographic systems such as GC and (HP)LC, the hyphenated techniques of GC-MS and LC-MS are increasingly becoming the confirmatory techniques of choice for residue analysis.

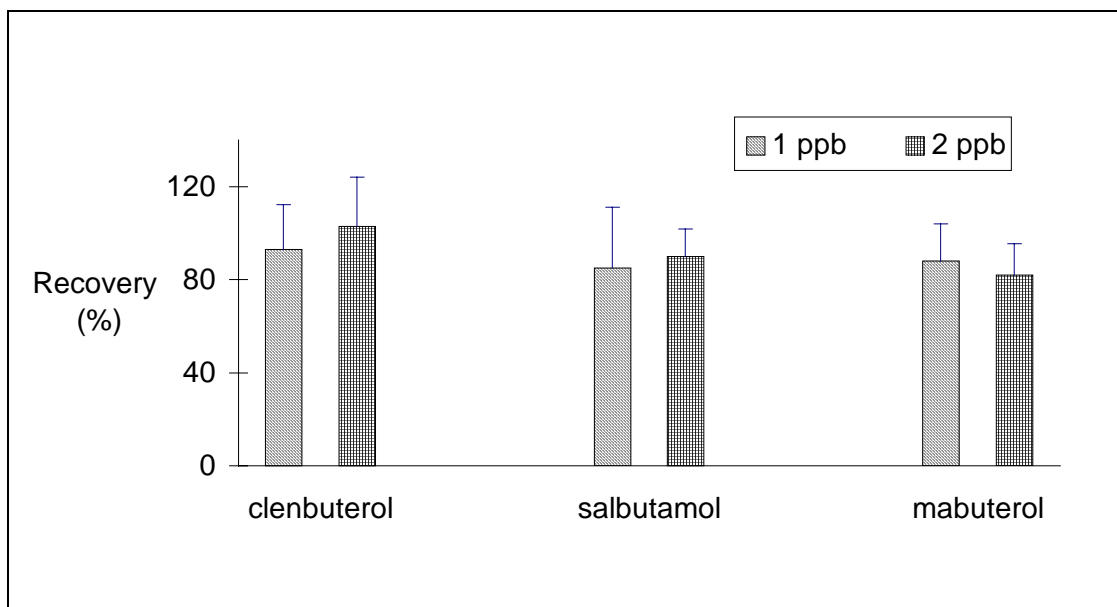
A method based on (a) protease digestion of tissue samples, adjustment of the supernatant to alkaline pH, extraction of residue with organic solvent and extract purification by SPE, and (b) mixed mode SPE for urine samples (as described above) were used to prepare sample extracts for GC-MS analysis. The method was used for determination of clenbuterol in liver samples and showed good quantitative performance (Figure 8). The methodology was used, also, for determination of clenbuterol and salbutamol in urine samples and performed well in a multi-laboratory study; Figure 9 shows the values obtained at The National Food Centre by comparison with values obtained at other European laboratories.

### **Summary**

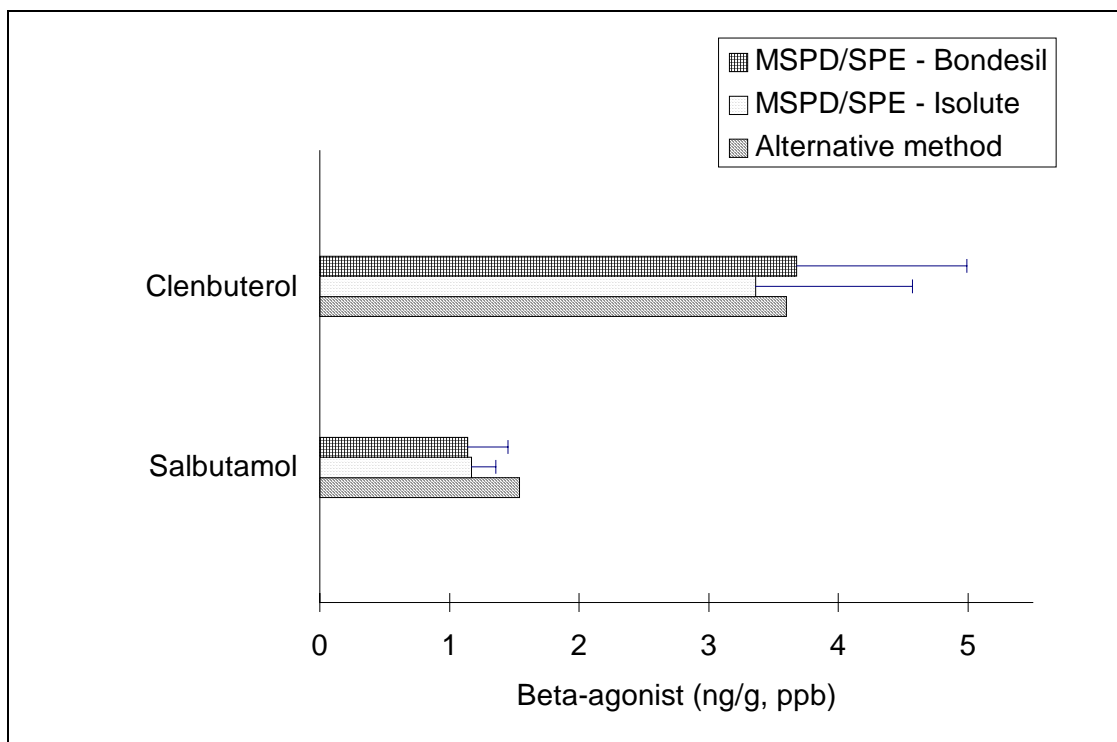
This research generated analytical systems for  $\beta$ -agonist residues in tissues and body fluids. Newer technologies, including MSPD, mixed mode SPE and mass spectrometry, have been applied to the determination of  $\beta$ -agonist residues. These technologies are used by The National Food Centre to provide analyses for this class of contaminants for the Irish beef industry.



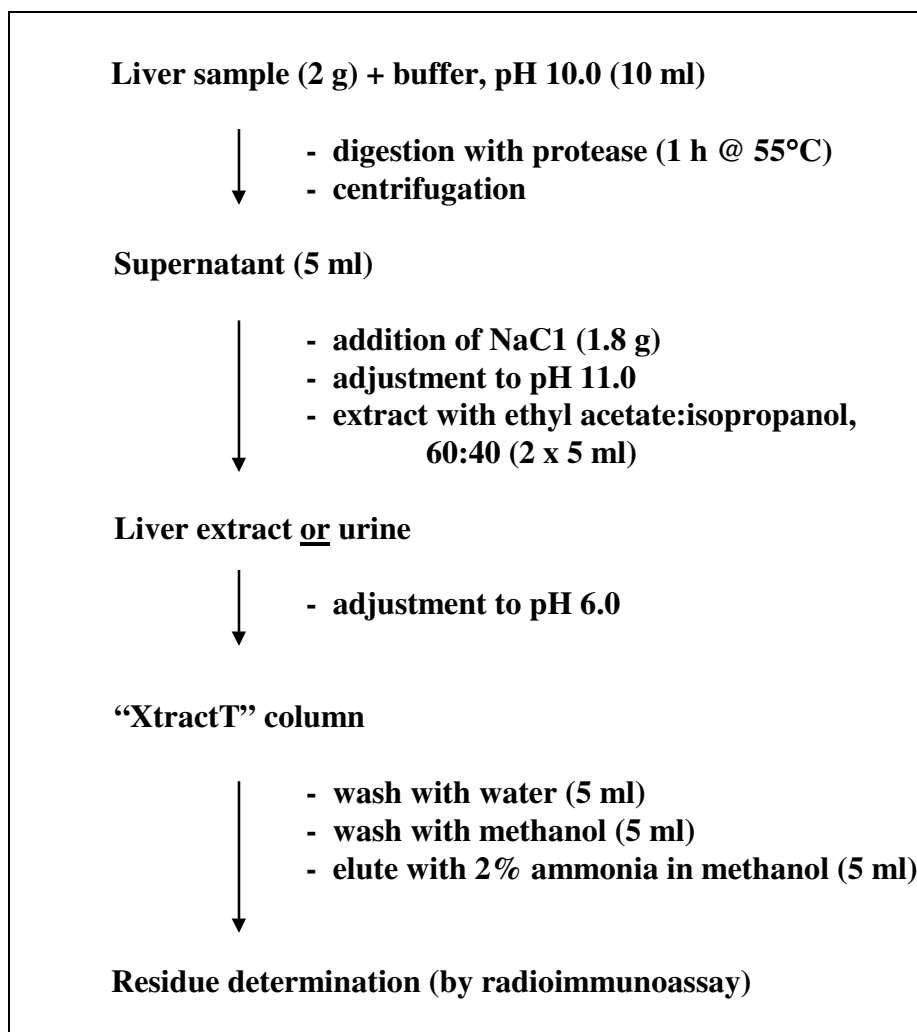
**Fig. 1:** New method for  $\beta$ -agonists in liver using matrix solid phase dispersion approach



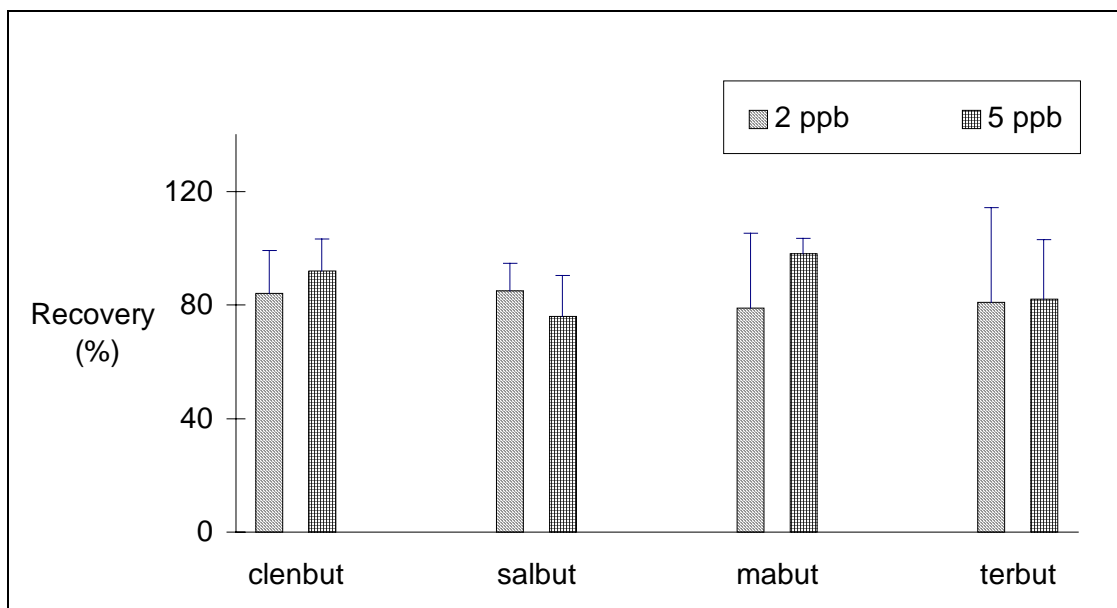
**Fig. 2: Recovery of  $\beta$ -agonists from liver by MSPD/SPE**



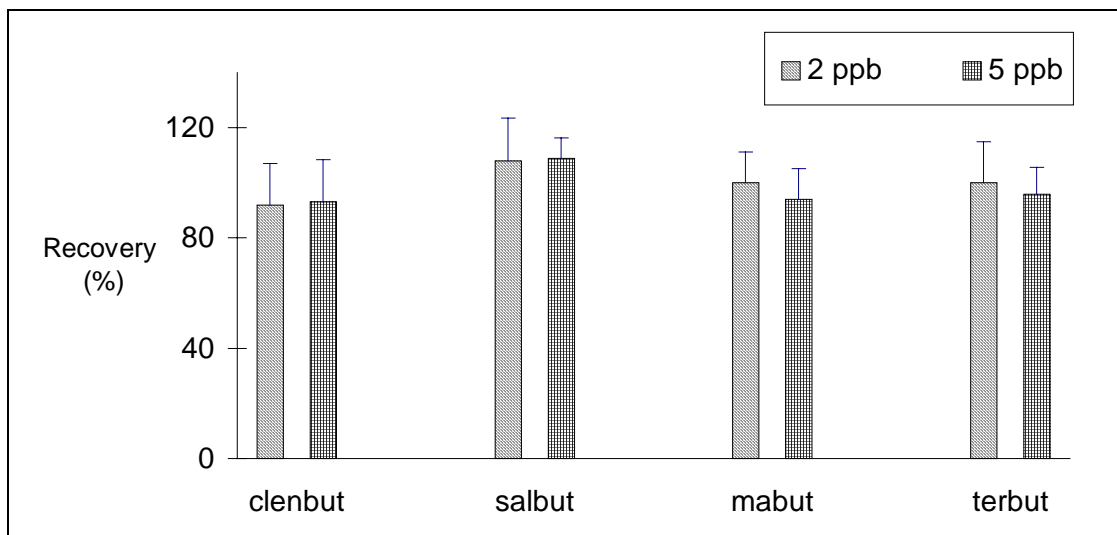
**Fig. 3: Comparison of results obtained by new method and alternative methods for assay of  $\beta$ -agonists in liver samples**



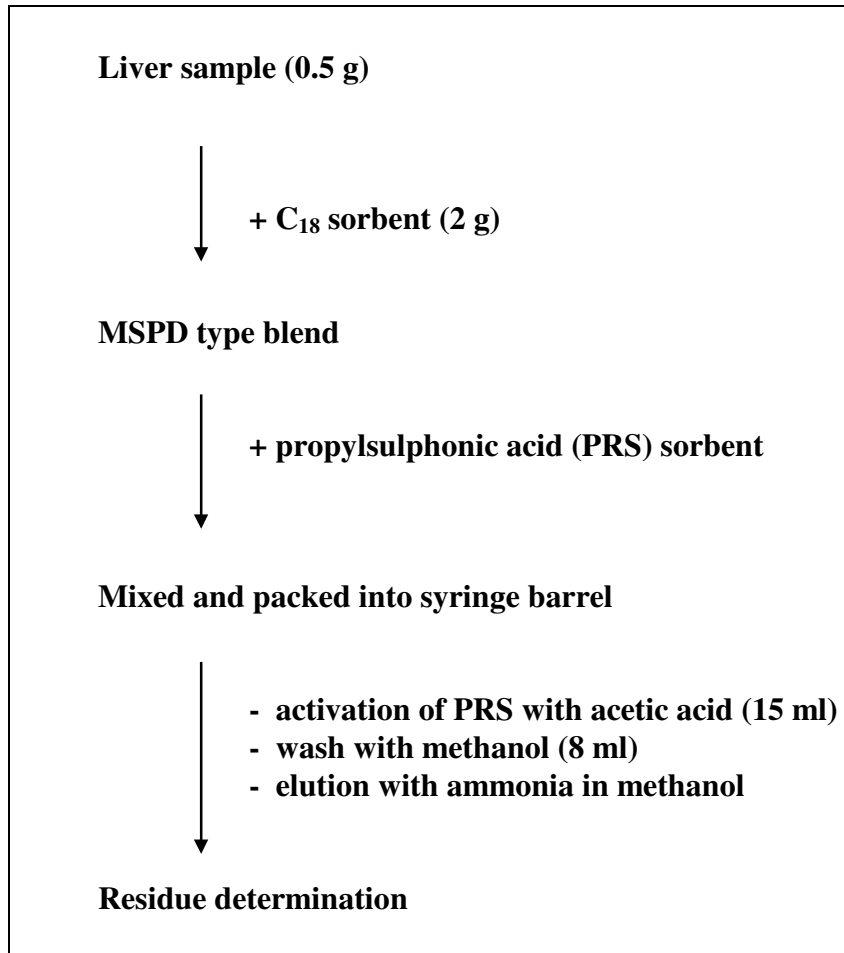
**Fig. 4: Mixed-mode SPE method for  $\beta$ -agonists in liver and urine**



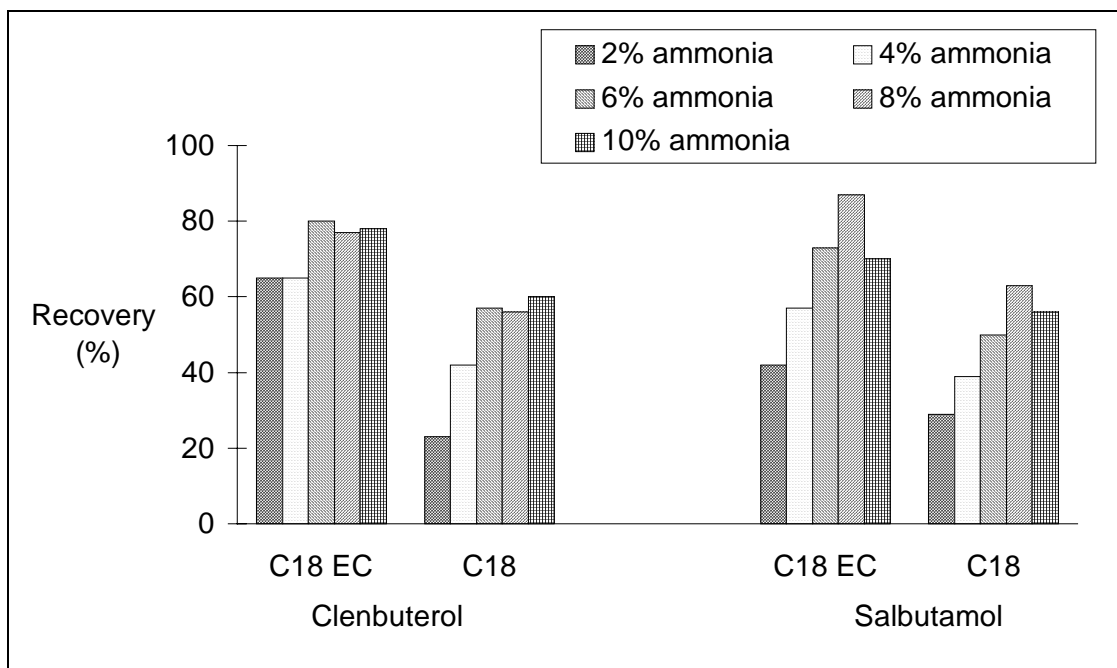
**Fig. 5a: Recovery of  $\beta$ -agonists from urine by mixed-mode SPE (clenbut - clenbuterol; salbut - salbutamol; mabut - mabuterol; terbut - terbutaline)**



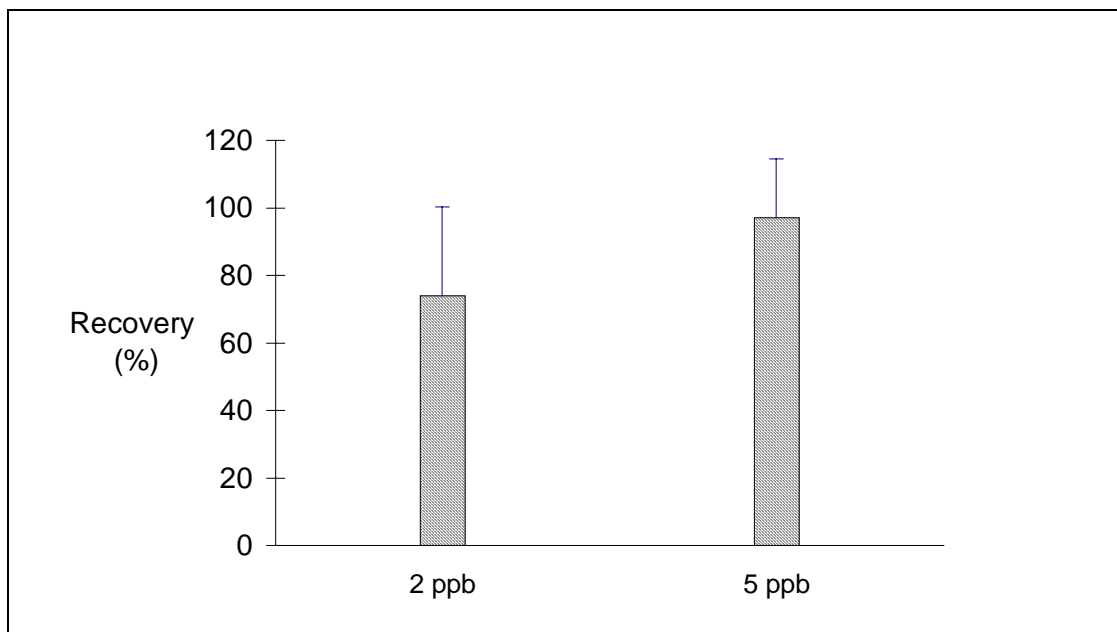
**Fig. 5b: Recovery of  $\beta$ -agonists from liver by mixed-mode SPE (clenbut - clenbuterol; salbut - salbutamol; mabut - mabuterol; terbut - terbutaline)**



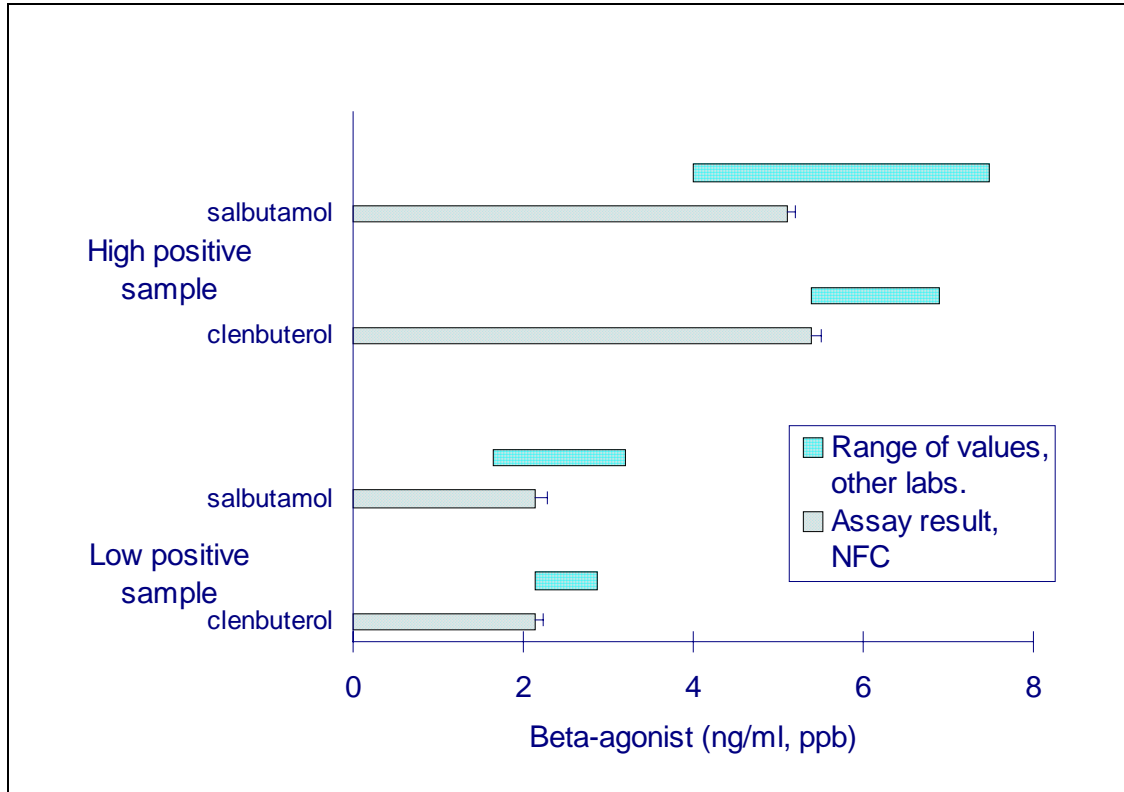
**Fig. 6: New method for  $\beta$ -agonists in liver using mixed-mode matrix solid phase dispersion approach**



**Fig. 7: Recovery of  $\beta$ -agonists from liver by mixed-mode matrix solid phase dispersion**



**Fig. 8: Recovery of clenbuterol from liver by gas chromatography - mass spectrometry**



**Fig. 9: Results of certification study for  $\beta$ -agonists in urine**

## ANALYTICAL METHODS FOR RESORCYLIC ACID LACTONES

Testing for the illegal use of the growth promoting agent, zeranol, in bovine animals is often undertaken through immunoassays of urine. Apparent positive results have been found by The National Food Centre in work for Irish meat companies and similar results have been reported from Northern Ireland (Kennedy et al., 1995), Sweden and New Zealand (Erasmuson et al., 1994). Further investigations in Northern Ireland have demonstrated that the source of these apparent positive results is the closely-related chemical, zearalenone, which occurs as a mycotoxin contaminant in animal feed. Both zeranol and its metabolites  $\beta$ -zearalanol and zearalanone, and zearalenone, and its metabolites  $\beta$ -zearalenol and  $\alpha$ -zearalenol, are members of the class of compounds known as resorcylic acid lactones. A method is required to identify the source of the residues - whether occurring naturally from mycotoxin contamination of feed or from growth promoter usage, through analysis for specific metabolites.

### **Zeranol/zearalenone metabolite analysis in urine**

Urine samples were extracted by hydrolysing the conjugated form of the residues to the free form and extracting the total residue with diethyl ether. The metabolites were separated on a reversed-phase HPLC system and fractions corresponding to the 6 metabolites were collected. The residue content in the fractions was determined by radioimmunoassay, but, because of the low cross-reactivity (5%) of the immunoassay antibody to  $\beta$ -zearalanol, determination of this metabolite was omitted from the procedure. The antibody cross-reactivities to the other metabolites ( $\beta$ -zearalanol, 29%; zeranol, 100%;  $\alpha$ -zearalenol, 54%; zearalanone, 100%; zearalenone, 53%) were sufficient to allow for quantitative determination. Adequate recovery values were determined for urine samples fortified with the five metabolites at a level of 3 ppb (Figure 10). The relatively high recovery obtained for some metabolites (e.g. zearalanone) and the relatively low recovery for other metabolites (e.g.  $\alpha$ -zearalenol) may be due to incomplete fractionation and/or inexact quantitation, caused by different cross-reactivities of the immunoassay antibody for the different metabolites.

The developed system was applied (Table 1) to the analysis of control urine samples from animals contaminated from growth promoter and from mycotoxin sources to establish which metabolites would be most suitable for use in identifying the source of residues (Fallon et al., 1996). Samples from a growth-promoter implanted animal showed detection of  $\beta$ -zearalanol, zeranol and zearalanone metabolites only; an implant of zeranol and the subsequent metabolism of the drug should not result in the formation of zearalenols or zearalenone. When applied to sample from an animal fed zearalenone-contaminated feed, the metabolite profile obtained showed the presence of zearalenone and  $\alpha$ -zearalenol, but also detection of  $\beta$ -zearalanol, zeranol and zearalanone, at lower levels. A number of test urines, obtained from suspect growth-promoter implanted animals were assayed by this procedure. The presence of  $\beta$ -zearalanol, zeranol, and zearalanone and the absence of any residues of  $\alpha$ -zearalenol or zearalenone indicates that these samples were from animals treated with the growth

promoter, zeranol. The different proportions of metabolites detected in samples may be due to metabolism variation in different animals and/or samples may have been taken at different times after implantation.

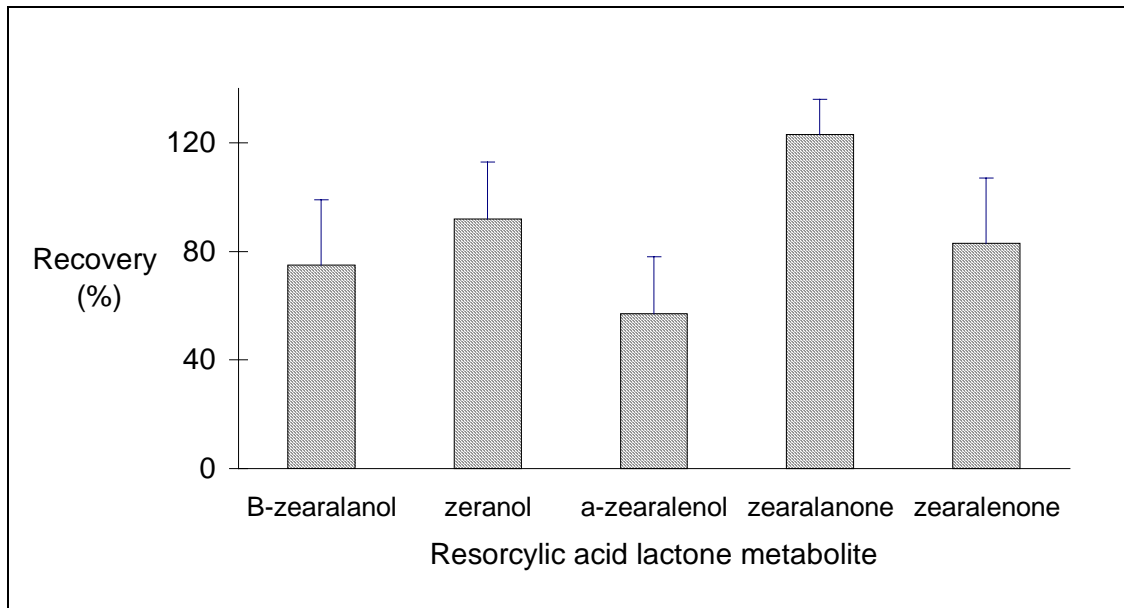
## Summary

Identification of the source of resorcylic acid lactones in bovine urine, whether originating from illegal use of the growth promoter zeranol or from feeding to animals of feed contaminated with the mycotoxin zearalenone, has been addressed by the development of these methods. Residue-positive samples, identified in a rapid screening procedure, may be re-analysed using HPLC fractionation and the metabolite profile identified. Where the reduced metabolites only are present, the most likely source of resorcylic acid lactones is from use of the growth promoter zeranol. Where the oxidised metabolites are present (with or without lower concentrations of the reduced metabolites), the source is most likely feeding of mycotoxin-contaminated feed.

**Table 1: Metabolites of zeranol/zearalenone in bovine urine**

Metabolite	Residue content (ng/ml, ppb)						
	Control urines		Test urines				
	Growth promoter treated animal	Mycotoxin fed animal	A	B	C	D	E
$\beta$ -zearalanol	2.6	< 0.5	4.2	3.5	8.9	6.2	4.2
zeranol	2.1	< 0.5	2.4	0.6	6.1	6.6	3.3
$\alpha$ -zearalenol	ND	0.7	ND	ND	ND	ND	ND
zearalanone	0.6	< 0.5	1.3	2.5	2.3	1.8	0.9
zearalenone	ND	1.1	ND	ND	ND	ND	ND

ND - no residue determined; < 0.5 - trace residue level determined, at less than 0.5 ng/ml



**Fig. 10: Recovery of resorcylic acid lactones from bovine urine by chromatography-immunoassay**

## **ANALYTICAL METHODS FOR ANTIMICROBIALS**

Antimicrobials are used widely in animal production for treatment of individual animals showing clinical signs of bacterial infection or for treatment of groups of animals, or even entire herds, to prevent or treat disease outbreaks. Usage of antimicrobials is particularly significant in intensive animal production systems, such as pigs and poultry.

Antimicrobials may be classified into different families of substances with similar chemical structure and properties, such as sulphonamides, tetracyclines,  $\beta$ -lactams, aminoglycosides, etc. Because related substances within a family have broadly similar activities in respect of their suitability for treating particular infections, analytical methods capable of detecting a family of antimicrobials are particularly beneficial. In this way food samples, such as meat, can be tested for a range of potential substances using a single analytical procedure.

The aim of this work was to develop systems for determining ranges of substances within particular families of antimicrobials, using efficient residue testing systems. Multi-residue procedures for sulphonamides, tetracyclines and  $\beta$ -lactams have been developed.

### **Sulphonamides by automated dialysis**

Sulphonamides are a group of drugs widely used in veterinary medicine for both the treatment and prevention of infectious diseases. Many of these compounds have a relatively long half-life and have potential toxic effects. Therefore, it is necessary to monitor meat products for drug residues of sulphonamides. The maximum residue limit (MRL) has been set at 100  $\mu\text{g}/\text{kg}$  (ppb) so as to guarantee the safety of animal products used for human consumption.

Automated dialysis, using the Gilson "ASTED" (Automated Sequential Trace Enrichment of Dialysates) equipment, was evaluated as a residue extraction procedure (McGrane et al., 1999). This technique, which involves a combination of dialysis across a semi-permeable membrane with trace enrichment of the dialysate on a microcolumn, is combined with automated HPLC analysis. The system was developed for the analysis of a range of sulphonamide antibiotic residues in edible tissues.

The procedure was evaluated by analysis of pork samples fortified with the range of sulphonamides at levels of 40 to 200 ppb. Mean recoveries were in the range 70 - 126% but chromatographic resolution for some drugs was affected by interferences from the sample matrix (Table 2). The results demonstrate that the ASTED system is a suitable analytical procedure for residues of sulphonamides in tissue, with a limit of determination of 20 ppb.

**Table 2: Recovery of sulphonamides from pork using automated dialysis**

Sulphonamide (fortified at 120 ppb)	Recovery (%) Mean $\pm$ SD, n = 5
Sulphadiazine	126 $\pm$ 27.8*
Sulphathiazole	86 $\pm$ 14.4*
Sulphapyridine	88 $\pm$ 10.7
Sulphamerazine	93 $\pm$ 8.1
Sulphamethizole	88 $\pm$ 7.8
Sulphamethazine	90 $\pm$ 9.9
Sulphamethoxypyridazine	82 $\pm$ 6.5
Sulphachloropyridazine	79 $\pm$ 9.4
Sulphafisoxasole	89 $\pm$ 5.9

\* matrix interference present

### **Sulphamethazine in pork products by thin layer chromatography**

The sulphonamide antimicrobial, sulphamethazine, has been used widely in pork production and manufacturers of pork products are required to demonstrate to their customers that these products are free of sulphamethazine residues at levels of concern. Typically, analysis of pork for sulphamethazine residues is undertaken using a HPLC method (Haagsma et al., 1985). While this method works well for meat, it is not suitable for analysis of products such as sausages and puddings due to the amount of co-extracted material which interferes with the chromatogram. A high performance thin layer chromatographic method was developed as an alternative (Research Report, 1996a).

Validation of the method was conducted on several products such as pork meat, white and black pudding, sausages and rashers. The validation consisted in fortifying 10g samples at 20 ppb, 50 ppb and 100 ppb sulphamethazine. The results for recovery of sulphamethazine in the various pork products are given in Table 3. The HPTLC method developed for the determination of sulphamethazine in animal tissue appears to be very suitable for a variety of products. Although variation in recovery is quite high, the absence of interferences in chromatograms presents a real advantage for the determination in comparison with the established HPLC method.

**Table 3: Recovery of sulphamethazine from pork products by a thin layer chromatography method**

Sample type	Fortification (ppb)	Recovery (%) Mean $\pm$ SD
Pork	20	66 $\pm$ 14.0
	50	73 $\pm$ 4.5
	100	93 $\pm$ 8.2
Sausages	20	76 $\pm$ 23.9
	50	81 $\pm$ 7.2
	100	83 $\pm$ 3.7
Rashers	20	87 $\pm$ 9.3
	50	86 $\pm$ 1.3
	100	83 $\pm$ 31.6
Pudding	20	79 $\pm$ 18.6
	50	77 $\pm$ 14.2
	100	83 $\pm$ 11.9

### **Tetracyclines by liquid chromatography - mass spectrometry**

Tetracycline antibiotics (oxytetracycline, tetracycline and chlortetracycline) are used in treatment of animals such as pigs. In 1996, residue-positive pork samples were found both by European (Debeuckelaere and Remy, 1996) and Irish (Kennedy and O’Keeffe, 1996) studies. Efficient screening, quantitative and confirmatory methods for tetracycline antibiotics are required.

A method for confirmation of tetracyclines in pork by liquid chromatography - mass spectrometry (LC-MS) was developed (Research Report, 1996b). The analysis was performed using a Micromass/VG Biotech Trio 2000 single quadrupole mass spectrometer with an atmospheric pressure chemical ionisation (APCI+) interface, with the molecular ion and one daughter ion for each tetracycline being monitored (Table 4).

**Table 4: Diagnostic ions and their proposed structures, as produced by liquid chromatography - mass spectrometry**

Antibiotic	Ion (m/z) and structure		
	[M+H] <sup>+</sup>	[-H <sub>2</sub> O] <sup>+</sup>	[-NH <sub>3</sub> ] <sup>+</sup>
Tetracycline	445	427	
Oxytetracycline	461	443	
Chlortetracycline	479/481*		462/464*

\* Chlorine 35/37 isotope peaks

### **β-Lactams by matrix solid phase dispersion**

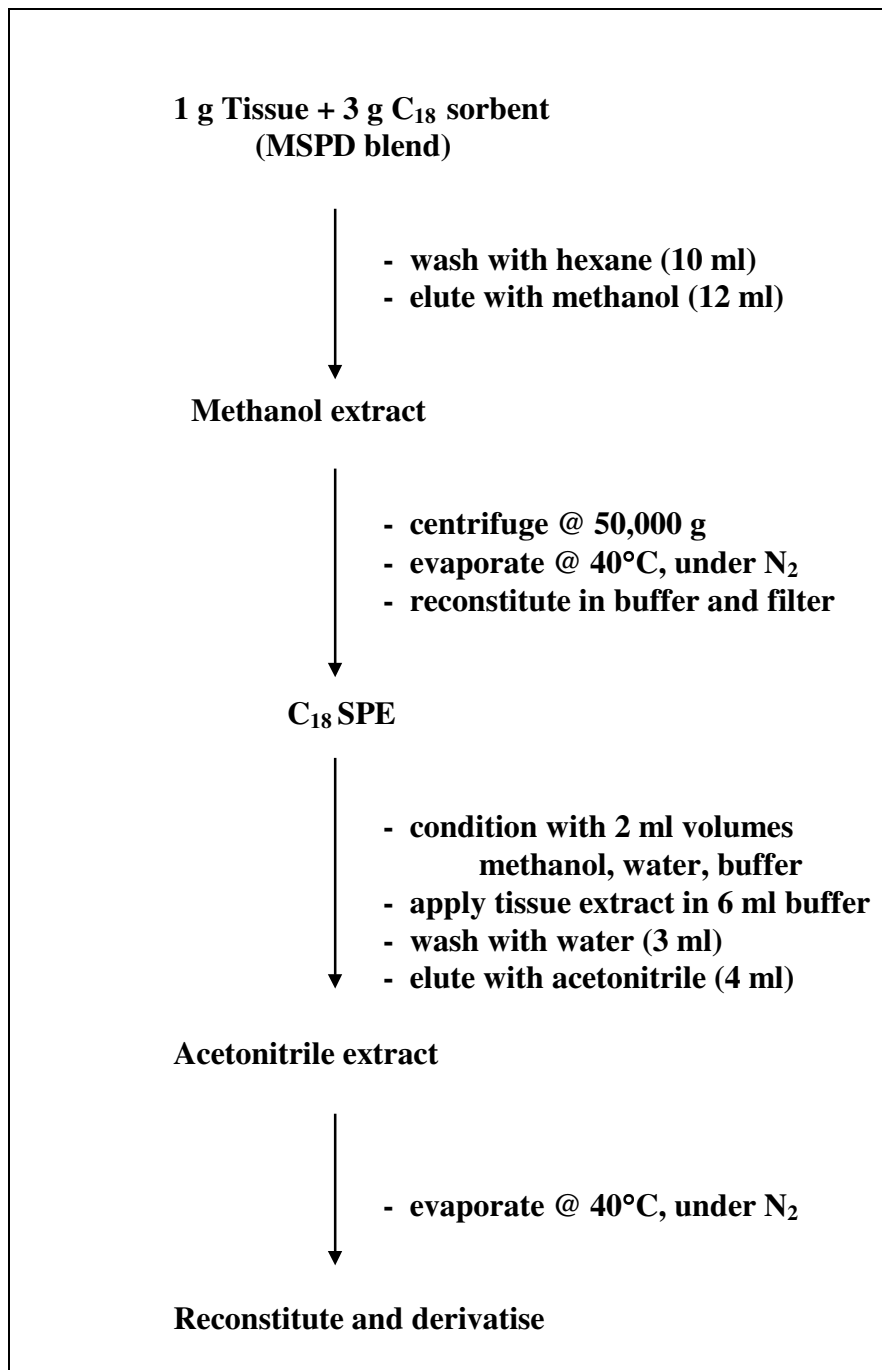
β-lactam, or penicillin, antimicrobials are an important family of drugs for which efficient multi-residue procedures are required. Matrix solid phase dispersion (MSPD) has proved to be a suitable extraction procedure for β-lactam antibiotics from pork (McGrane et. al., 1998). Following residue extraction by MSPD and extract clean-up by SPE (Figure 11), the extracted residues are derivatised by incubation with triazole and mercuric chloride, separated on a phenyl column by gradient elution at 30°C and detected by UV at 325 nm.

Various SPE systems were evaluated for the clean-up but the C<sub>18</sub> SPE was found to be most satisfactory. Mean recovery for β-lactam antibiotic fortified samples (200 ppb) varied from 62 to 76% for ampicillin, penicillin G, cloxacillin and dicloxacillin. At lower levels of fortification (e.g. 40 ppb) more variable recovery was observed.

This method is unsuitable for the other important β-lactam antimicrobial, amoxicillin, because this drug is not separated from matrix components. However, alternative HPLC conditions may allow for determination of amoxicillin. Use of an internal standard and/or matrix-containing standard curve might be useful procedures to compensate for low recoveries. The advantage of this method is that it allows for determination of the four β-lactam antibiotics in a single, relatively straightforward procedure.

## **Summary**

Analytical methods have been developed for different classes of antimicrobial residues in meat and meat products, including MSPD, automated dialysis, HPLC, HPTLC and LC-MS. The methods developed are suitable for multi-residue analyses at levels below the maximum residue limits (MRLs) for these antimicrobials in meat.



**Fig. 11: MSPD method for  $\beta$ -lactam antimicrobials in pork**

## CONCLUSIONS

- The matrix solid phase dispersion technique has been applied successfully, for the first time, to the determination of  $\beta$ -agonist residues in bovine liver and is shown to be an efficient and robust method.
- Sorbents of differing chemistries have been used to develop “mixed mode” methods for  $\beta$ -agonists in urine and liver samples. These methods, applied either in solid phase extraction or matrix solid phase dispersion modes, give high recovery of  $\beta$ -agonist residues from samples and are efficient extraction and clean-up procedures.
- Gas chromatography - mass spectrometry is used to confirm the presence of  $\beta$ -agonist residues in samples screened as positive by immunoassay techniques.
- The “immunogram” technique has been used to address an important commercial problem - to discriminate between residue-positive samples containing resorcylic acid lactone metabolites, either as being from anabolic agent (zeranol) treated animals or from animals fed the mycotoxin zearalenone.
- Sulphonamide antimicrobial residues may be determined in meat using the automated dialysis technique and in pork products using thin layer chromatography.
- Liquid chromatography - mass spectrometry is used to confirm the presence of tetracycline antibiotics in pork.
- A method based on matrix solid phase dispersion has been developed for multi-residue analysis of penicillins in meat.

## REFERENCES

- Boyd, D., Shearan, P., Hopkins, J.F., O'Keeffe, M. and Smyth, M.R. 1993. Application of matrix solid phase dispersion for the determination of clenbuterol in liver samples. *Analytica Chimica Acta*, **275**, 221-226.
- Boyd, D., O'Keeffe, M. and Smyth, M.R. 1994. Matrix solid phase dispersion (MSPD) as a multiresidue extraction technique for  $\beta$ -agonists in bovine liver tissue. *Analyst*, **119**, 1467-1470.
- Boyd, D., O'Keeffe, M. and Smyth, M.R. 1995. Matrix solid phase dispersion linked to solid phase extraction for beta agonists in liver samples: An update. *Analytical Proceedings*, **32**, 301-303.
- Collins, S., O'Keeffe, M. and Smyth, M.R. 1994. Multi-residue analysis for beta-agonists in urine and liver samples using mixed phase columns with determination by radioimmunoassay. *Analyst*, **119**, 2671-2674.
- Collins, S., O'Keeffe, M., Calverley, R. and Smyth, M.R. 1996. Studies on the development of mixed-mode matrix solid-phase dispersion for the extraction of  $\beta$ -agonist residues from liver. *Proceedings EuroResidue II, Veldoven, The Netherlands*, 340-345.
- Debeuckelaere, W. and Remy, R. 1996. Research on the presence of antibiotic residues in meat originating from the fifteen E.U. member states. Association des Consommateurs - Test-Achats S.C., Contract No. B5-1050/95/000130, 28 pp.
- Erasmuson, A.F., Scahill, B.G. and West, D.M. 1994. Natural zearanol ( $\alpha$ -zearalanol) in the urine of pasture-fed animals. *Journal of Agricultural and Food Chemistry*, **42**, 2721-2725.
- Fallon, A., O'Keeffe, M., Gosling, J.P. and Kane, M. 1996. Development of a HPLC-immunoassay system capable of identifying the source of zearanol metabolites (Abstract). *Irish Journal of Agricultural and Food Research*, **35**, 227.
- Haagsma, N., Nooteboom, R.J., Gortemaker, B.G.M. and Maas, M.J. 1985. *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, **181**, 194.
- Kennedy, D.G., McEvoy, J.D.G., Blanchflower, W.J., Hewitt, S.A., Cannavan, A., McCaughey, W.J. and Elliott, C.T. 1995. Possible naturally occurring zearanol in bovine bile in Northern Ireland. *Journal of Veterinary Medicine (Series B)*, **42**, 509-512.
- Kennedy, O. and O'Keeffe, M. 1996. Generation of data on the residue status of Irish foods (Abstract). *Irish Journal of Agricultural and Food Research*, **36**, 226.

McGrane, M., O’Keeffe, M. and Smyth, M.R. 1999. The analysis of sulphonamide drug residues in pork muscle using automated dialysis. *Analytical Letters*, **32** (3), (in press).

McGrane, M., O’Keeffe, M. and Smyth, M.R. 1998. Multi-residue analysis of penicillin residues in porcine tissue using matrix solid phase dispersion. *Analyst*, **123**, (in press).

Martinez-Navarro, J.F. 1990. Food poisoning related to consumption of illicit  $\beta$ -agonist in liver. *Lancet*, **336**, 1311.

Pulce, C., Lamaison, D., Keck, G., Bostvironnois, C., Nicolas, J. and Descotes, J. 1991. Collective human food poisonings by clenbuterol residues in veal liver. *Veterinary and Human Toxicology*, **33**, 480-481.

Research Report, The National Food Centre. 1996a. HPTLC method for sulphamethazine in pork and pork products. p. 59.

Research Report, The National Food Centre. 1996b. Tetracycline antibiotic residues determination by LC-MS. p. 64.

Remy, R. and Debeuckelaere, W. 1994. Residues of growth promoting substances in meat. Association des Consommateurs - Test-Achats S.C., Contract No. B5-1050/93/006893, 71 pp.