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Review

Analytical strategies for the screening of veterinary drugs and their residues in edible products

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Abstract

The development of analytical strategies for the regulatory control of drug residues in food-producing animals is discussed. Analytical methods for the determination of veterinary drugs in edible products are based on microbiological, immunochemical and physicochemical principles. Because of complexity of biological matrices such as egg, milk and meat, well designed, and often sophisticated, off-line or on-line sample treatment procedures are essential, especially when utilising physicochemical multi-residue screening procedures. Since large series of samples have often to be analysed, automation is increasingly becoming important. Confirmation of the identity of drug residues and validation of the analytical results implies the use of adequate analytical methods. In its turn, this requires well established criteria for those methods and/or equivalent reference methods.

Contents

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1. Introduction

In modern agricultural practice, veterinary drugs are being used on a large scale. The majority of these drugs is administered as feed additives or via the drinking water in order to prevent the outbreak of diseases or to improve the growth of the animals. Besides that, therapeutic drugs are given in case of disease, for drying-off purposes, or for the prevention of losses during transportation. In the Netherlands, where the legislation and use of veterinary drugs are regulated both on a national and an European Community/Union (EC/EU) basis, more than 3000 veterinary drug preparations have been submitted for registration. They contain more than 200 different active substances. Most of these products are claimed to have antibacterial or antiparasitic potency.

Unlike the situation with human drugs or veterinary drugs for pets, the use of veterinary drugs for food-producing animals such as poultry, lactating cows and swine can affect the public health and the international trade of food products because of the presence of residues of the drug, or of its metabolites, in edible products (milk, eggs, body tissue after slaughter). Depending upon the time-span between the administration of the drug and the collection of the animal product (withdrawal period), drug-related residues may be present in these products. Other factors which determine the occurrence of residues are the route of administration, contamination of feeds or water, the physicochemical properties and metabolism of the drug, and the physical condition of the animal. The amount of drug residue that can be regarded negligible should be based on toxicological considerations. In practice, unfortunately such internationally harmonized maximum residue levels (MRLs) often do not exist. Instead, in many cases the limits of detection of the available analytical methods determine whether residue levels are considered violative or not. For those classes of drugs which are suspected carcinogens or mutagens, no residues should be detectable. Recently, maximum residue levels for various veterinary drugs have been established within the European Union. In the Appendix (Table A2), the MRLs for some veterinary drugs and their residues in different kind of matrices of different kind of food-producing animals are given.

Obviously, the availability of sensitive and accurate analytical methods to monitor animal products for the presence of residues of veterinary drugs, is essential. In addition, pharmacokinetic and metabolism studies which indicate the time-course of drug depletion and the presence of relevant metabolites, are of the utmost importance for the establishment of a governmental residue policy.

Only a few years ago, the meat and milk control of residues of veterinary drugs in most countries was based almost exclusively on microbiological methods. For eggs, sensitive and selective methods were completely absent. The microbiological methods allowed one to detect a rather broad range of antimicrobials, but with strongly varying limits of detection (LOD) typically ranging from 2 μg/kg to 10 mg/kg. Extensively used groups of drugs such as the sulphonamides, the suspected mutagens or carcinogens of the nitrofuran, nitroimidazole and quinoxalin classes, the toxic antimicrobial chloramphenicol, tranquillizers and the antiparasitic drugs could not be determined with adequate sensitivity.

Recently, several sensitive, accurate and automatable non-microbiological analytical methods for the determination of residues of relevant classes of veterinary drugs in milk, eggs and animal tissues have become available. These methods are suitable for routine monitoring and surveillance programmes and for pharmacokinetic experiments, and allow the determination of relevant metabolites. Suitable spectrometric confirmation methods, which are useful for unambiguous identification are also increasingly being developed.

In the present review, the analytical aspects of the regulatory control of veterinary drug residues are discussed. First, aspects such as the extensive range of veterinary drugs presently in use, the stability of residues in biological matrices, and the role of metabolism will be discussed. As regards the last aspect, the metabolic pattern can vary between animal species, and within one animal the ratio of the various metabolites may vary between individual body tissues or body fluids. Besides, metabolites can be (at least) as potent, or toxic, as the parent compound. A major factor in drug residue analysis is the extremely complex matrix. Therefore, the gross composition and relevant properties of urine, plasma, milk, meat and egg will be summarized, and the essential sample treatment procedures necessary prior to introduction of such samples into modern analytical (chromatographic or spectrometric) equipment will be briefly discussed.

The major part of the review is dedicated to a description of various options for the development of analytical strategies for the control of veterinary drug residues. Multi- and single-residue screening procedures can be based on biological activity or on physicochemical interactions. For the latter category which requires more or less sophisticated instrumentation, automation and selectivity are key factors. Modern off-line and on-line sample treatment procedures will be reviewed, including the use of post-column reaction detection in LC. For confirmation of residues, spectrometric techniques providing direct structural information should preferably be used. Identification using diode-array UV-Vis (DAD), Fourier-transform-infrared (FT-IR), nuclear magnetic resonance (NMR) and mass spectrometric (MS) detection will be discussed. Finally, the validity of the analytical results will be shown to be largely determined by the quality of the methods used and the quality assurance procedures within the control laboratory.

2. Veterinary drugs and their residues in biological matrices

2.1. Specific aspects of the determination of drug residues

The main objectives of the regulatory control of residues of veterinary drugs are: (i) to assure a safe and wholesome food supply, and (ii) to take regulatory action after identification of adulterated products. In order to do this, complex matrices such as milk, meat or eggs have to be monitored–often on a routine basis–for a large number of physicochemically and structurally highly different compounds at concentration levels ranging from 1 to 1000 μg/kg. Furthermore, if regulatory action has to be taken, the results obtained with the control methods will have to be highly reliable and unequivocal.

2.2. Range of compounds and metabolism

In Western Europe several hundreds of active veterinary drugs are commercially available, and

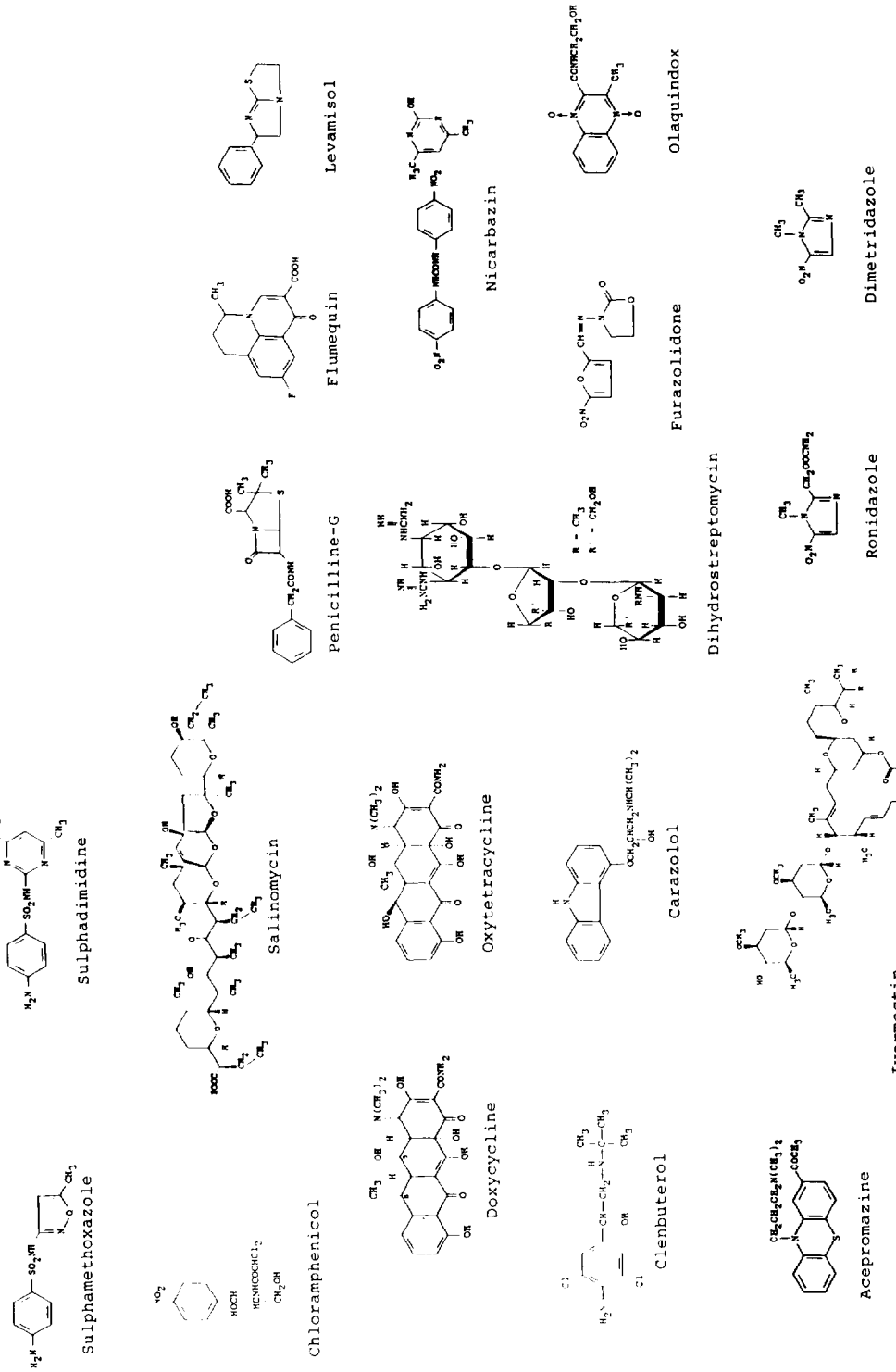

Fig. 1. Structures of typical representatives of the main classes of veterinary drugs.

at least seventy-five of these are being used more or less extensively for food-producing animals. The structures of a number of veterinary drugs that will often be quoted in the present review, and which are typical representatives of the various classes of drugs are shown in Fig. 1. Most of these drugs are metabolized in the body in order to produce more water-soluble compounds which should be more readily excreted. The number of metabolites may range from zero–as is the case for e.g. aminoglycosides [1]–to more than twenty–as for example with chlorpromazine [2]. The metabolic process is generally divided in two phases. In phase I, the drug is enzymatically oxidized, reduced or hydrolysed. In phase II, the parent drug or the phase I metabolite is chemically transformed to a water-soluble conjugate mainly by sulphatation, glucuronidation, acetylation or conjugation with glycine. In some cases, the metabolites are more active or more toxic than the parent drug. An example of the formation of active metabolites is depicted in Fig. 2, which shows the metabolic conversion of the pro-benzimidazole anthelmintic febantel, to fenbendazole, which in turn is oxidized to oxfendazole [3]. Actually, all three compounds are also individually on the market as anthelmintics. Another striking example is enrofloxacin which main deethylated metabolite is ciprofloxacin, a potent antimicrobial used in human medicine [4]. An example of a toxic metabolite is the desoxy metabolite of carbadox [5]. The immuno-allergic properties of β-lactams are related to, mainly, a number of major metabolites which act as haptens [6].

A complicating factor is that the biotransformation of a drug may vary substantially between animal species, as was clearly demonstrated for sulphadimidine [7]. For those drugs where information is available with regard to relevant metabolites–e.g. sulphonamides [7], nitroimidazoles [8], chloramphenicol [9] and carbadox [5]–the analytical methods should be capable of detecting both the metabolites and the parent compound. For a number of other drugs, e.g. nitrofurans [10] and coccidiostats, the metabolic pattern in relevant animal species has not (completely) been elucidated. In such cases, the control of edible products has necessarily to be focused on the analysis of the parent compound. The presence of structurally related but unknown metabolites may interfere in the determination of the drug and stresses the need for selective confirmation procedures.

2.2.1. Stability of residues in the biological matrix

A number of studies have been performed regarding the stability of nitrofurans [11], chloramphenicol [11–14], sulphadimidine [12,13,15], and selected antibiotics [13], after cooking, curing, fermentation or (freezing) storage of edible products. These studies indicate that, in most cases, part of the parent drug has vanished as a result of the treatment, but they do not give any insight into the nature of the decomposition products formed. The stability of levamisole [16], quinolones [17], sulphadimethoxine [18], and carbadox [5,19] in edible products have recently been discussed. An overview has been published by Haagsma [20]. Whereas the former two studies only describe a decrease of the drug residue after food processing and preparation, the latter four provide some more insight in the degradation products that are formed during storage and/or processing and may be present in edible products. In general, the identification and characterization of decomposition products formed during the processing of residue-containing food products presents a challenge to both analytical chemists and toxicologists.

Summarizing the above we can conclude that, theoretically, residues from several hundreds of drugs and drug-related compounds may be present in a random sample obtained from food-producing animals, and that control methods should be capable to detect, quantify and identify these compounds.

2.3. The biological matrix

The determination of drug residues is generally performed in the edible product, which mostly is milk, animal tissue, or eggs. For screening purposes, however, it may be attractive to ana-

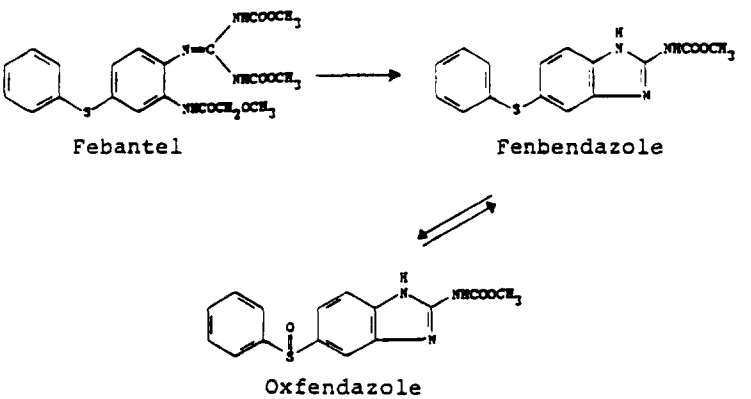

Fig. 2. Biotransformation of the pro-benzimidazole anthelmintic drug febantel to the benzimidazole anthelmintics fenbendazole and oxfendazole.

lyse faeces or body fluids which are easily available–i.e. urine, bile or plasma–and in which the drug concentration is elevated. To illustrate the difficulties that can be encountered during analysis, some typical properties of the most important matrices will be discussed.

2.3.1. Urine

Urine normally does not contain proteins and lipids and so does not readily produce emulsions or foams when extracted with an organic solvent. However, the composition of urine varies from species to species and also depends on the diet of the animals. An illustration is the colour which may vary from dark amber to pale yellow. The pH can range from 4 to 9. Upon standing, urine becomes more alkaline by loss of carbon dioxide, which results in precipitation of phosphates and organic salts. It is therefore essential that urine samples are buffered to a uniform pH before analysis, and that the analytical method is validated for a variety of urine samples obtained from different animals and different species and various periods of the day.

Because many drugs are excreted in urine as conjugates, and the free, i.e. the non-conjugated drug fraction shows a large intra- and inter-animal variation, it is customary to treat urine samples with a combination of β-glucuronidase/arylsulphatase to release the conjugated drug fraction. This will improve the analyte detectability. The added enzyme itself and the decomposition products formed during the deconjugation step may give rise to an increase in chromatographic interferences. As an example of the latter aspect, the influence of enzymatic deconjugation on the LC determination of residues of chloramphenicol in milk is shown in Fig. 3 [91]. Similar effects are obtained for urine samples, although the extent which interference occurs of course also depends on experimental conditions such as detection mode and pretreatment procedure.

2.3.2. Plasma

Plasma contains lipids, salts, enzymes (esterases) and, unlike urine, a substantial amount of proteins (about 7%). Despite its complex nature, the variation in the composition of plasma within an animal species is small, except for the lipid content which is diet-dependent. The pH of plasma always is about 7.4. Because of the high affinity between some drugs and plasma proteins, one can differentiate between the "free" or unbound and "total" concentration of the drug. Binding proteins in plasma include albumin, α-acid glycoprotein, lipoproteins and τ-globulins [21]. For drugs which are highly ($>90\%$) protein-bound, the free drug concentration self-evidently is very low.

Although the free fraction of the drug can be considered the physiologically active portion

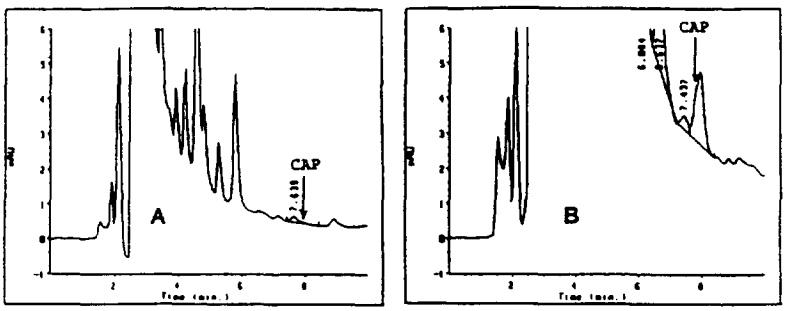

Fig. 3. Typical example of increased matrix interferences as a result of enzymatic deconjugation. The LC–UV determination of chloramphenicol (CAP) in milk is shown. (A) Blank milk, (B) blank milk treated with β-glucuronidase/arylsulphatase. Conditions: enrichment column (10 × 2.1 mm I.D.) Bondapak C_{18}/Corasil; eluent acetonitrile–sodium acetate; analytical column 5 μm Chromspher C_{18} (200 × 3 mm I.D.); DAD detection 225–400 nm [91].

which governs the residue level in tissues, for screening purposes the total drug content is determined in most cases. In a number of immunochemical screening tests however, the plasma samples are analysed as such, which may imply that the free fraction is determined only. With both total and free-fraction analyses it is assumed that the bound fraction is a constant for an individual animal and even for one animal species. After establishment of the percentage protein binding, the result of the assay is then supposed to correlate with the tissue residue level. In practice, however, the free fraction is to some extent concentration-dependent, decreasing at lower drug levels [22], and is greatly influenced by the pathological state of the animal [21]. For some classes of drugs, the tissue/plasma distribution ratio of the drug is much higher than 1, which results in higher tissue levels than expected on the basis of the plasma concentration. This is fro instance the case for the third-generation fluoroquinolones [23,24]. In contrast to therapeutic drug monitoring, in a residue screening programme one can compensate for this variation by using a method establishing a sufficiently low limit of detection for a drug in plasma.

2.3.3. Milk

Milk can be considered as an emulsion of fat droplets in an aqueous milk plasma. However, the membrane of such a fat particle is much more complex than an ordinary emulsion globule membrane, and consists of a mixture of water, proteins, lipids, enzymes, minerals, phosphatides and other compounds. Furthermore, the milk plasma is not homogeneous and contains a colloidal solution of globular proteins, a dispersion of lipoproteins and a dispersion of casein micelles. The former two classes of proteins are the serum proteins.

The casein micelles consist of casein proteins, inorganic salts, water and enzymes. The micelles can be precipitated by acidification to pH 4.6 or heating above 120°C. The serum proteins are not precipitated at this pH but become insoluble upon heating above 80°C and precipitate on the casein micelles. The gross composition of milk is given in the Appendix (Table A1) [25]. The composition of milk is influenced by genetic factors, the physiological condition of the animal, climate and the diet. Apart from that, the composition may change upon storage as a result of pasteurization, oxidation, enzymatic conversions and growth of microorganisms. Milk contains a number of natural, microorganism-inhibiting substances [25]. These include immunoglobulins which may agglutinate Gram-positive bacteria (IgM-lactenins L_1 and L_3), the enzymes peroxidase and lysozyme, and lactoferrine, which inhibits the analytically important bacteria *Bacillus stearothermophilus* and *Bacillus subtilis*. All these naturally occurring inhibiting substances can be inactivated by heating, although

this may also affect the stability of, for instance, β-lactam antibiotics [26].

In the udder alveoli, drug residues are transported from the blood stream to the milk by passive diffusion. In principle, non-dissociated apolar compounds are most easily transported [27], and it is unlikely that polar drug conjugates (i.e. glucuronides) will occur in milk.

Because of the physicochemically different phases in milk, drugs will sometimes be distributed unevenly and may remain predominantly in one phase after, e.g. acidification or decreaming. Table 1 shows some data on the distribution of a number of antimicrobials over cream, casein and whole milk, as established in radiolabelled studies with goats by Ziv and Rasmussen [27]. The distribution depends both on the residue concentration range and the route of administration. The latter phenomenon can be explained by the trapping of the more lipophilic compounds during the formation of fat globules in the milk-secreting cells in the udder alveoli. When the drug is added after the fat globule has been formed–as is the case with intramammary injection, but also when spiking a sample!–the enrichment in the fat globules apparently does not take place to the same extent. The distribution of a drug residue over the various phases should be established for each individual drug.

As in plasma, drugs can be bound to proteins in milk. However, unlike plasma, milk is consumed; it is therefore essential to determine the total drug content in order to establish whether the milk is contaminated.

2.3.4. *Animal tissue*

Muscle is composed of muscle fibres, various types of connective tissue, adipose tissue, cartilage and bone. After visible fat is removed, which is normally done prior to analysis, the gross composition of muscle is shown in the Appendix (Table A1) [28]. Sarcoplasmic proteins such as myoglobin and glycolytic enzymes, are soluble in water while the myofibrillar proteins, i.e. myosin and actin, are soluble in concentrated salt solutions. The connective tissue proteins, collagen and elastin, are insoluble in both solvents.

In contrast with the situation for milk where in essence only cow milk is analysed, with meat the drug residues have to be determined in samples of different origin, viz. pork, poultry, veal, cattle and lamb meat. Apart from differences between muscle tissue from various parts of one animal, there are qualitative and quantitative differences in composition between animal species. There-

Table 1
Distribution of a number of radiolabelled antimicrobials in various milk compartments[a] [27]

Drug	Administration form[b]	Ratio cream/whole milk	Ratio casein/whole milk
Benzylpenicillin	I. Mamm.	0.3–0.5	1.0–1.1
	I. Musc.	1.0–2.1	0.8–3.2
Spiramycin	I. Mamm.	0.4–0.9	1.5–22.8
	I. Musc.	0.9	22.6
Chloramphenicol	I. Mamm.	1.1–2.3	2.2–24.8
	I. Musc.	7.4–8.1	22.4–24.5
DH-Streptomycin	I. Mamm.	0.3–0.6	1.3–400
	I. Musc.	1.0	260
Tetracycline	I. Mamm.	0.4–0.7	2.0–22.4
	I. Musc.	1.1–3.2	25.6–726

[a] The concentration ratios presented in the table are mean values obtained with four animals, based on radiolabelled experiments. They are concentration-dependent as can be seen from the difference between the first (high-drug level) and the second (low-drug level) figure given with each range.

[b] I. Mamm. = intramammary infusion; I. Musc. = intramuscular injection.

fore, analytical methods will always have to be tested on material from each individual species, because differences in coloured components such as myoglobin in poultry and beef, fat composition and the presence of species-specific proteins, may influence the analytical recovery and can cause interferences during analysis. In a study on the determination of residues of nitrofuran drugs in edible products [29] in which an aqueous extraction was used, an adequate analytical recovery (>75%) was obtained for furazolidone after spiking chicken and veal calf meat, whilst only about 10% recovery was obtained after spiking pork meat. In the latter case the recovery could markedly be improved by the addition of about 25% of acetonitrile during extraction. Possibly, furazolidone is strongly bound to a pork-specific protein [26].

Covalent binding of drug residues to macromolecular tissue components, which results in non-extractable residues, has extensively been studied, mainly through radiolabelling studies and bioavailability experiments. Nitroimidazole, nitrofuran and benzimidazole drugs have been shown to give more or less persistent "drug-like" non-extractable residues with a varying bioavailability after oral administration to rats [10,30–32].

Liver and kidney are often used as target matrices because of the elevated residue levels. However, especially liver contains very active metabolic enzyme systems such as the cytochrome P_{450} complex and reductase activity. This enzymatic activity may lead to post-mortem in vitro metabolism of drugs, as is the case in the rapid and complete inactivation of chloramphenicol and carbadox in liver and kidney [5,19,33,34]. Cytochrome P_{450} activity can be inhibited by the addition of piperonyl butoxide prior to analysis. The recovery of chloramphenicol in bovine liver homogenate samples containing piperonyl butoxide was found to be 2-fold higher (60% rather than 30%) than in untreated liver homogenate samples [33]. The post-mortem inactivation of drug residues may lead to the philosophical question as to whether it is relevant to know the residue level at the time of slaughter when it can be anticipated that the concentration of active components will be strongly diminished at the time of consumption or further technological treatment. This is certainly the case for many of the β-lactam antibiotics [26].

2.3.5. Eggs

An egg consists of two distinct units with a very different composition. In the Appendix (Table A1), the gross composition of egg white (albumen) and egg yolk (ovum) is given [35,36]. The albumen constitutes about 60% of the total egg weight and essentially is a colloidal dispersion of 10% globular glycoproteins in water. The ovum is a more complex system; it contains particulate granules, which consist of a mixture of high-density lipoproteins, phosvitine and low-density proteins, which are suspended in a micellar protein solution. The fatty acid composition of the yolk lipids (e.g. the linoleic acid content) depends to some extent on the composition of the hen's diet. The content of yolk pigments and, therefore, the colour of the egg, also depends on the specific layer's feed. In practice, the feed given to a hen largely determines the, mainly chromatographic, interferences observed in the analysis of eggs.

The high lipid content of the yolk makes it an apolar medium, while the albumen is polar. One can therefore expect differences in the concentrations of polar and apolar drugs in the two compartments. Table 2 shows several examples which demonstrate this behaviour after oral dosage to laying hens. The ionophoric coccidiostat monensin is more polar than the analogues salinomycin and narasin. Other drugs giving elevated residue concentrations in yolk are the coccidiostats amprolium [40] and nicarbazin [41].

If drug residues have to be determined separately in yolk and egg white, it is advisable to separate these compartments immediately after laying because of reported diffusion from yolk to egg white [42,43]. As was the case with urine, it is also necessary to buffer an egg sample prior to analysis, as the pH of albumen may vary between pH 7.6 and 9.2 with the pH of yolk being about 6.5 [35,36].

Table 2
Distribution of ionophoric coccidiostats [37], flumequine [38], sulfaquinoxaline and nitrofurans [39] in egg yolk (y) and egg white (ew) after oral medication to laying hens

Drug	Medication		Mean level (μg/kg)		Ratio y/ew
	Period (days)	Level (mg/kg)	Yolk	Egg white	
Monensin	7[a]	110	100	150	0.7
Narasin	7[a]	70	1000	250	4.0
Salinomycin	7[a]	60	1500	50	30.0
Flumequine	10[b]	90	400	2000	0.2
Sulfaquinoxaline	7[a]	100	1300	3700	0.35
Nitrofurazone	7[a]	100	5000	3000	1.70
Nitrofurantoin	7[a]	100	<	100	
Furaltadone	7[a]	100	2000	900	2.20

[a] Feed medication.
[b] Water medication.
<, not detectable above 1 μg/kg

2.4. Sample treatment

Microbiological inhibition assays and also other assays based on biological activity in general (see next section) do not require sample treatment because of the selectivity and sensitivity of the detection principles and the screening purpose for which the assays are being used. In contrast, sample treatment is of major importance when physicochemical techniques are used. The main objectives of sample treatment are (i) removal of macromolecules and other matrix constituents that may either adversely affect the chromatographic system or interfere with the detection, and (ii) the enrichment of the analytes in order to achieve the required low limits of detection. Sample treatment procedures that are normally used in veterinary drug residue analysis are summarized in Table 3. The different modes of sample treatment and the procedures which are generally used for each of the biological matrices will be discussed in this section.

Apart from sample treatment, residue analysis almost invariably involves a (chromatographic) separation-cum-detection system. Nowadays, LC is by far the most extensively used chromatographic technique in veterinary drug residue analysis, with high-performance(HP)TLC/TLC

Table 3
Sample treatment procedures in veterinary residue analysis

Homogenization	
Enzymatic digestion	
Purification and enrichment	–off-line liquid–liquid extraction –immunoaffinity clean-up –on-line or off-line solid-phase extraction –on-line dialysis –on-line or off-line size-exclusion chromatography –column-switching LC
Filtration and centrifugation	–removal of solids
Derivatization	–pre- or post-separation labelling or analyte conversion

in second place. This is primarily due to the fact that most of the target analytes are medium-to-highly polar. The use of GC has been reviewed by Petz [44]. For confirmation purposes (which will often require derivatization), capillary GC coupled with MS detection plays an important role. Although a technique such as adsorptive stripping voltammetry can offer selectivity and sensitivity without physical separation of the analytes [45,46], direct measurement of the analytes, even after derivatization, is only adequate for group identification and may easily give rise to false positives [47,48].

General aspects of sample clean-up and detection in chromatographic veterinary drug residue analysis have been discussed by Shaikh and Moats [49], Petz [44,50], and Haagsma [15]. Physicochemical methods have also been reviewed [51], and special studies have been devoted to chloramphenicol [52], aminoglycosides [53] and ionophoric antibiotics [54].

2.4.1. Urine and plasma

Because of the low viscosity of buffered and filtered urine, it can be directly applied to off-line solid-phase extraction (SPE) cartridges [2,55–57], as well as to short on-line LC enrichment columns filled with either non-selective alkyl-bonded silica or polymer-based materials, or more selective ion-exchange, metal-ligand or immunoaffinity materials [58,59–61]. The monitoring of drugs in biological fluids has been reviewed [2,62] and overviews have been presented regarding the use of proteolytic enzymes, aqueous and organic solvents and SPE materials for the removal of proteins and the release of protein-bound drugs [62–65]. The direct injection of plasma onto LC systems, using micellar systems [66], hydrophobic stationary phases [64,67] and size-exclusion or immunoaffinity columns has also been reported [68,69]. The use of direct injection techniques has been reviewed [70].

2.4.2. Milk

Because of the heterogeneous composition of milk, in most cases it is necessary to perform an off-line liquid–liquid or solid-phase extraction [52,71–76] to remove proteins. However, when using on-line dialysis (see below and [77,78]) this is not necessary. It is even possible to repeatedly inject decreamed milk directly onto a short enrichment column coupled on-line with an LC system. As an example, Fig. 4 shows LC chromatograms on the determination of diaminodiphenylsulphone (dapson) and its two main metabolites [91]. Another possibility is the use of off-line immunoaffinity columns to selectively enrich drugs in milk, as was demonstrated for chloramphenicol [79]. Recently, a direct immunofiltration dipstick assay was developed for the determination of chloramphenicol in milk [80]. Immunochemical assays for screening of milk for a number of antibiotics and sulphonamides are available [81]. Ultrafiltration can also be used for sample treatment as was shown for ceftiofur and ceftiopirin in milk [82,83]. In all cases it should be checked whether drug–protein binding has been completely broken.

2.4.3. Animal tissue

Muscle and organ tissues will always require some form of sample pretreatment to obtain a

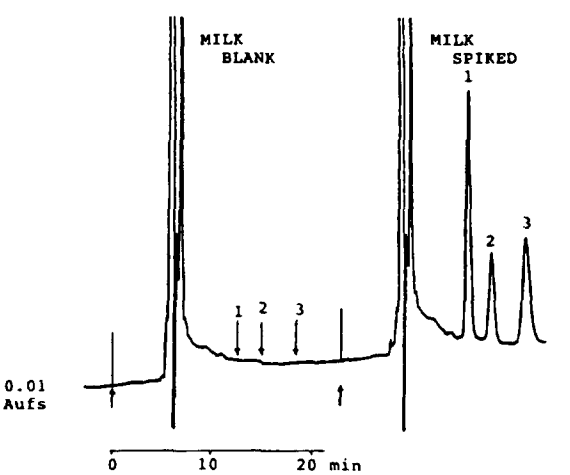

Fig. 4. Direct injection of 200 μl decreamed milk onto a column-switching LC system. The sample was spiked with 100 μg/l each of dapson (1) and its monoacetyl (2) and diacetyl (3) metabolites. Conditions: enrichment column (60 × 3 mm I.D.) Bondapak/Corasil C_{18}; eluent acetonitrile–water (20:80, v/v); analytical column LiChrosorb C_{18} (200 × 3 mm I.D.); UV detection at 292 nm [91].

homogeneous liquid phase which contains the drugs of interest and is sufficiently free from interferences. The extent of sample treatment depends on the analytical goal (screening/confirmation), the scope of the method (single/multimethod), the physicochemical properties of the drug (e.g. protein binding), and the selectivity and sensitivity of the detection mode. Very simple sample treatment is used in the microbiological screening of antibiotics [84], where a paper disc is inserted in the kidney. After absorption of the renal pelvis fluid, it is placed on an agar plate which is supplemented with trimethoprim and utilizes *B. subtilis* as bacterial test system. Another example of simple clean-up, which is allowable because of selective and highly sensitive detection, is the immunochemical screening of chloramphenicol [9]. In this test polyclonal antibodies directed against chloramphenicol are immobilized in wells on the cards. Enzyme-labelled chloramphenicol competes for the available binding sites and a color substrate indicates the presence of chloramphenicol. For a number of veterinary drugs screening tests are available which require limited sample clean-up [81,85]. In other cases, combinations of homogenization, purification and enrichment, or filtration and centrifugation steps (Table 3) in the off-line or on-line mode are often performed [50,86–91]. The use of enzymatic digestion of tissue prior to extraction [92] is attracting renewed interest [93].

2.4.4. Eggs

Because of the high level and great variety of proteins and lipids in eggs, binding of drug residues to these compounds can occur. The removal of proteins and fat, with the subsequent release of the drug presents considerable experimental problems because of the formation of emulsions and foams upon extraction with an organic solvent. Acetonitrile is now considered to be the best extraction solvent [50]. Aqueous precipitation of the proteins is difficult because of the wide range of isoelectric points of the protein classes (pH 4.5–11.0) [94]. A combination of heat and low pH will generally precipitate all proteins, at the risk, however, of the inclusion of drug residues during precipitation. The lipoprotein granules can be removed by centrifugation [95]. Another approach to sample preparation is to coagulate the proteins by the addition of salts, thereby forming a smooth suspension which can be subjected to a SPE procedure [8]. Finally, diluting the sample with saline diminishes the tendency of proteins to coagulate and allows the on-line dialysis of egg extracts [29,91,96], provided the drug–protein binding is low or is sufficiently reduced by the dilution step.

Similar to milk, eggs contain the microorganism-inhibiting enzyme lysozyme, which should be inactivated or removed prior to microbiological detection by heating at 65°C [95,97], or partitioning with isooctane [37].

3. Analytical strategies in regulatory control

3.1. General remarks

In essence, there are two types of regulatory residue programmes, viz. (i) programmes where the animal or the product is held up pending the result of the analysis and (ii) control programmes that are used to monitor the residue status of food of animal origin, without rejection of the specific product. In both cases, suspected samples should be efficiently separated from the bulk of negatives. The latter category can then be released while the, often few, positive samples can be examined further to establish whether the product contains violative residue levels. In its simplest form, a control programme therefore consists of a single analytical method that enables one to screen large numbers of samples for the presence of a variety of residues, and simultaneously to identify and quantify the residues that have been found. Unfortunately, ideal methods such as are depicted in Fig. 5 are not encountered in the real world. Therefore, in practice a control programme is often divided into a screening phase and a confirmation phase, which each use appropriate analytical methods. A screening method should allow the detection of all suspect samples, preferably using a simple, routinely applicable procedure. A confirmation

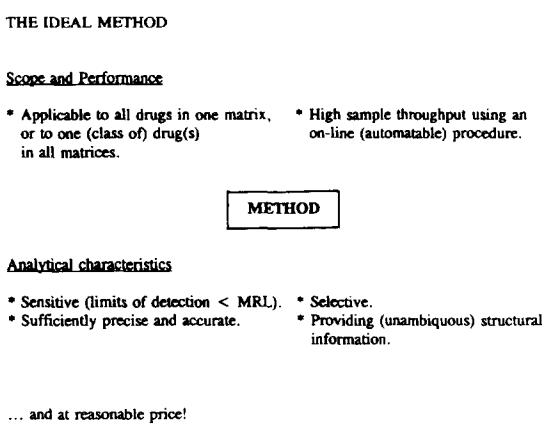

Fig. 5. The ideal analytical method.

method should unequivocally establish the identity of the residue. During the regulatory control of non-prohibited drug residues, reliable quantification has to be carried out at an appropriate stage. Quantification should enable one to reliably establish whether the residue concentration exceeds the maximum residue level (MRL).

When developing or selecting analytical procedures for residue control programmes, one has to take into account a number of aspects, some of which are governed by external–e.g. political or organisational–factors. Some of these are summarized in Table 4. A relevant example of the impact of the items listed in Table 4 is given in Ref. [9], where an analytical strategy for the regulatory control of chloramphenicol in meat and milk is described.

In the next sections various options for the screening and confirmation of residues of veterinary drugs in edible products will be discussed. In the section dealing with physicochemical multi-residue methods, special attention will be paid to the automation of the sample treatment of biological samples, and to the selectivity enhancement that can be introduced during this step.

3.2. Screening

In this paper, a screening method is defined as the first procedure that is applied to sample analyses, the purpose being to establish the presence or absence of residues of veterinary drugs. This procedure should be as simple as is possible. Still, it may be rather complex, due to, e.g. the properties of the drugs of interest or the desired limit of detection, and, in certain cases, will provide (semi)quantitative next to the qualitative information.

Table 4
Factors influencing the set-up of an analytical strategy

Available laboratory facilities (peripheral or central laboratory)	-personnel -equipment
Purpose and scope of control	-surveillance -multi-residue screening
Number of samples per day	
Available methodology	-development -implementation
Available finances	-use of MS -development
International agreements	-methods -products
Maximum residue levels	-banned drugs -changes with time

There are two main options when carrying out a screening programme, viz. (i) to use multi-residue methods aimed at the determination of groups of drugs having similar characteristics [98,99] and (ii) to use single-residue methods applicable to one specific drug. These options will be discussed below.

3.2.1. Multi-residue screening methods

Veterinary drugs show a large variation in molecular structure and, consequently, in physicochemical properties and biological activity. Because the aim of the control of residues is to prevent that residues in the food will exert an undesirable effect on humans, it would be elegant to use this biological effect as detection principle in the screening procedure. Obviously, a prerequisite is that the effect is rapid, reproducible, and can be detected with great sensitivity. Generally, with the undesired effects of veterinary drug residues, one can differentiate between long-term toxic effects (carcinogenicity, mutagenicity), pharmacological effects (sedation, antiparasitic action), antimicrobial effects, allergenic effects, and technological effects (dairy industry). In principle, the MRLs for the drug are based on the absence of these effects.

In reality, only the antimicrobial activity can be measured fast, simply and with high sensitivity and can therefore be used as a direct detection principle [100]. Long-term toxic effects are extremely difficult to mimic in a simple test at low drug concentrations. To a lesser extent this also holds for the pharmacological effects, although a test for ionophoric activity has been reported for residues of the coccidiostat salinomycin [101]. The technological effect of veterinary drugs in milk (influence on starter cultures) primarily is an antimicrobial problem and therefore can be tested on the basis of antimicrobial activity [102]. As a consequence, all drugs that do not possess antimicrobial activity should be screened on the basis of other types of physiological reactivity, e.g. using immunochemical or receptor assays, or on the basis of their physicochemical properties.

3.2.1.1. Microbiological multi-methods

An analytical chemist dealing with the development of state-of-the art residue methods will probably consider the microbiological inhibition assays used for the detection of antimicrobials to be inadequate because they are neither very reproducible nor highly selective, have a strongly varying sensitivity for the various drugs, do not detect inactive metabolites and are rather slow (from 3 to 16 h). The opinion of a residue control officer may, on the other hand, be that the inhibition assays are excellent screening methods because they are not too selective and therefore are able to simultaneously detect many drugs, are often more sensitive than any other method, are simple to perform, do not require sophisticated equipment, and are completed within a day. Actually, both opinions are correct and numerous modifications of inhibition assays have, therefore, been tested to either enhance the selectivity or broaden the scope.

Methods using *B. stearothermophilus var. calidoactis* as test organism, which are often used in milk control are extremely sensitive for penicillins (with LOD, about 5 μg/l). However, they generally are more than 100-fold less sensitive for other commonly used antibacterial agents such as macrolides, sulphonamides, tetracyclines and chloramphenicol [102–106]. In view of this, it is even questionable whether these methods can be considered as true multi-methods. Even though improvements have been reported [107], in practice more than 90% of the positive findings with these types of methods refer to a penicillin [108]. A number of rapid on-the-farm tests to check whether milk is contaminated with, mainly, penicillins is commercially available [81,109,110]. Most of these tests can only be used on bulk tank milk because the milk of individual animals which are often sampled after drying-off or after mastitis treatment, contains high concentrations of natural inhibitory substances which give a false positive result. Table 5 lists the test for milk tanker residue control accepted by the Food and Drug Administration (FDA) [111]. These tests have been validated by FDA and the producers.

Table 5
Milk drug residue screening test detection levels as assessed by FDA in 1993[a]

Screening test	Drug (at tolerance or safe level indicated)					
	Amoxicillin (10 ppb)	Ampicillin (10 ppb)	Ceftiofur (50 ppb)[b]	Cephapirin (20 ppb)	Cloxacillin (10 ppb)	Penicillin (5 ppb)
Charm II tablet competitive assay	10	9	25	4.5	70	4.8
Charm Farm test	10	10	25	20	40	5
Charm II tablet sequential assay	10	8	23	4.5	50	4.8
Charm II tablet Transit test	10	9	13	4.5	80	4.8
Charm Rapid inhibition test	4.5	4.5	50	16	25	3
Charm I/Cowside II test	10	10	40	8	50	4.8
Charm II tablet quantitative assay[c]	1.4	1.5	2	1	10	1
Charm *B.stearothermophilus* tablet disk assay	10	6.5	75	11	48	4.8
Cite probe β-lactam test[c]	12	12	50[d]	8	100	5
Delvo test P[c]	8	10	50	8	30	3
Delvo-X-Press	10	10	10	10	50	5
LacTek B-L	10	8	ND	16	8	5
LacTek CEF	ND	ND	50	ND	ND	ND
Penzyme III test	8	10	80	8	80	5
Penzyme milk test	8	10	95	8	80	5
Snap test	10	10	50[e]	8	50	5

[a] Parts per billion (ppb) which can be detected by test 90% of the time with 95% confidence. Precise 90/95 levels were not normally determined for sensitivities significantly above nor significantly below the tolerance or safe level.
[b] Parent drug.
[c] Test added to original list released by FDA on October 22, 1993.
[d] Test produced six of six positive results at 10 ppb ceftiofur.
[e] Test produced five of six results at 10 ppb ceftiofur.
ND = not detected.

Most of the microbiological tests in meat control use muscle or kidney as target tissue. The obvious advantage of analysing muscle is that this is the edible part of the animal for which MRLs have primarily been established [see also Appendix (Table A2)]. Another advantage is that false positives due to naturally inhibiting substances are not likely to occur [34]. A disadvantage is that a variety of microorganisms have to be used to meet the MRLs for the commonly used antimicrobials [112], as is the case with the so-called four-plate test which initially was proposed as a routine screening method in the EC. However, the test is laborious and relatively expensive [84] and has now been suggested as an EC reference method for antimicrobials. Test systems using kidney as indicator tissue for muscle have the advantage of a better sensitivity, because with most antimicrobial residues the highest free drug concentrations are found in kidney and renal pelvis. Therefore, less microorganisms will be require than when testing muscle. However, there are also drawbacks such as the different ratios of residue levels in kidney and muscle of diseased animals, the production of false positives results and the accumulation of aminoglycosides [34,84,112]. For example, high aminoglycosides concentra-

tions have been observed for weeks in kidneys, whereas the drug residues in muscle tissue decreased below the minimum detectable level within one day [84]. With aminoglycosides, the incidence of false negative results observed in muscle tissue, is highest in diseased animals.

In the Netherlands a test system with improved sensitivity for sulphonamides obtained by the addition of trimethoprim and sodium chloride was developed which uses only one microorganism, *B. subtilis*. Renal pelvis fluid is the target substrate. The test is simple, does not give false positives and–because of the high ratio of residue concentrations in pre-urine compared to muscle–can ensure that a negative test result implies that residue levels in muscle are below the limit of detection of the EC four-plate test [34,84,112–115]. More recently, a simple and rapid ATP/bioluminescence test has been developed with which it is possible to obtain a screening result within 4 h as compared with 18 h for conventional microbiological assays [116]. Very few microbiological methods have been published for eggs [97,117,118]. False positives caused by lysozyme represent a major problem.

3.2.1.2. Microbial receptor-assay multi-method

A multi-residue method for antimicrobials in milk has been developed which is based on the binding reaction between functional groups of the drug and receptor sites on added microbial cells (Charm-Test II, [119]). Cells from two different organisms provide the binding sites for seven families of drugs, viz., β-lactams, tetracyclines, macrolides, sulphonamides, aminoglycosides, novobiocin and chloramphenicol. The test employs ^{14}C- and ^{3}H-labelled drugs to compete for the binding sites. The reported limits of detection range from below 5 μg/l for sulphonamides, β-lactams and chloramphenicol, to 10 μg/l for tetracyclines and 30 μg/l for aminoglycosides [103,119–122]. The test can be regarded as a rather complex and sensitive screening method complementary to the microbiological control methods. It can also be used as a confirmatory test for samples which have been found positive with a microbiological screening method. Compared with physicochemical screening methods for the seven drug families mentioned above, the Charm-Test II is simple, rapid, sensitive and inexpensive. However, reported false positive results and limits of detection which are above safe or maximum residue levels, indicate the necessity to confirm presumptive Charm II test results [123].

3.2.1.3. Physicochemical multi-methods

Since physicochemical methods for residue analysis require extensive sample clean-up, they can no longer be performed on a routine basis in simply equipped laboratories. In a previous section, general aspects of the sample treatment of biological liquids and edible products have been discussed. In this section we shall primarily discuss the possibility of on-line (automatable) operations and the selectivity of the total analytical procedure. Next to the frequency with which false positives occur, these are aspects of major importance for a screening method and largely determine its applicability for routine analysis.

As has been stated above, screening procedures should be simple. Yet we have seen that biological matrices are rather complex and that different sample treatment procedures are required to prepare a suitable final extract. Automation is therefore highly desirable. For TLC analyses, no automated on-line sample preparation techniques for biological samples are operational. For GC, the use of a continuous-flow system containing a liquid membrane, which is directly coupled to the GC part of the system has been described for the determination of amines in urine [124]. Another promising approach is the on-line trace enrichment of analytes from aqueous samples or extracts, using a short LC-type pre-column packed with, e.g. C_{18}-bonded silica or a polymeric sorbent, and coupled on-line with a capillary GC via a retention gap [125].

Nowadays, commercially available equipment allows the fully automated solid phase extraction, either off-line or coupled on-line to an LC instrument [126–129]. The direct injection of biological fluids into a column-switching (CS) LC system is also possible [58–60,66–70,130]. Main

applications of column-switching techniques to drug analysis have been reviewed [65]. Today, the development of intelligent and versatile autosamplers allows one to perform essential steps such as homogenization and liquid–liquid extraction in a fully automated mode [129]. This is particularly useful where a derivatization step has to be included. Evaporation and centrifugation of inhomogeneous samples is still difficult to automate without using a rather expensive, and still not very versatile, robotic system. A key factor in these systems is the extraction with an organic solvent, which requires the quantitative transfer of the analyte-containing apolar phase, and the complete removal of water to allow successful evaporation [50,131]. Furthermore, emulsions may be formed [50].

Aqueous extraction would seem to circumvent most of the above practical problems and can facilitate the automation of sample treatment. For relatively polar analytes such as veterinary drugs, aqueous extraction can, therefore, be an attractive option. Lipids and other apolar compounds present in a biological sample will not be co-extracted and denaturation of proteins depends on the pH that is selected. Furthermore, as a result of the addition of the extraction solvent the sample is diluted which will decrease drug–protein binding. This means that, after centrifugation, the analytes are in the phase that can be directly applied to a suitable SPE system [8,9] or–with, e.g. milk–can be injected onto a column-switching LC system which allows automation. Reliable automated LC determination generally requires removal of the co-extracted proteins. This can be done on-line using dialysis [29,78,95,132–134] or size exclusion chromatography [68,135–137]. An overview of published procedures for dialysis combined on-line with precolumn/analytical column LC is presented in Table 6.

It is a drawback of aqueous extraction that interfering polar components may be co-extracted. This means that, depending upon the chromatographic and/or spectroscopic properties of the drugs, additional selectivity has to be introduced before the final detection step. Another factor which has to be carefully considered for each individual drug is its extractability by the very polar water or buffer solutions used. Most drugs, even relatively apolar ones such as chloramphenicol, some sulphonamides and nitroimidazoles which are only sparingly soluble in water, can be quantitatively extracted from matrices such as milk, egg, and animal tissue at the proper pH. However, aqueous extraction of the apolar coccidiostats salinomycin, narasin and monensin from eggs, was not successful [26]. Strongly protein-bound drugs sometimes are not fully extracted. In all cases, the aqueous extraction method should be compared with a method employing a conventional "organic" extraction, using both spiked and naturally incurred samples.

The selectivity of a multi-residue screening method can be defined as its potential to discriminate between the analytes under investigation and other substances which are present in the sample, mainly matrix constituents. The maximum number of peaks that can be resolved in a typical TLC, LC or capillary GC run (about 20, 50 and 200, respectively [145]), is distinctly lower than the number of residues of veterinary drugs and of (main) matrix interferences that can be present. In other words, additional selectivity has to be introduced into the assay. If the analytes of interest can be selectively detected because they show long-wavelength (above about 350 nm) UV-Vis absorption, as do the nitrofurans and quinoxalines [29,146,147], display native fluorescence, such as the β-blocker carazolol [148], the (fluoro)quinolones [149–152], and some sulphonamides [153], or show antimicrobial activity such as the ionophoric antibiotics (bioautographic detection [37,54]), one can achieve low limits of detection (< 10 μg/kg) while using rather simple clean-up procedures.

A modest degree of selectivity is obtained during clean-up using liquid–liquid extraction (solvent polarity, pH) or SPE (alkyl-bonded silica-based, or polymer-based packing) [58,60,154]. However with a multi-method with which several (groups of) drugs with different physicochemical properties have to be determined, the uniform sample clean-up approach will inevitably result in differences in analyte

Table 6
Fully automated methods for residue analysis of veterinary drugs using on-line dialysis combined with LC

Drug	Matrix	Detection limit (μg/kg)	Detection method[a]	References
Nitrofurans	egg/meat/milk	1–10	UV, 365 nm	29
Amprolium	egg/meat/feed	5	Flu., ex. 365 nm, em. >470 nm post-column deriv. with hexacyanoferrate	40
Chloramphenicol	milk	3	UV, 280 nm	77
Oxytetracycline	egg/milk/ muscle/liver	1 3–8	Flu., ex. 358 nm, em. 460 nm addition of NaOH, irradiation at 366 nm	78
Sulphonamides	egg/meat/milk	25	Vis., 450 nm, post-column derivatization with dimethylamino-benzaldehyde	96
Flumequine	egg/meat/milk	5–10	Flu., ex. 240 nm, em. >370 nm, post-column derivatization with sulphuric acid	38,132
Flumequine + oxolinic acid	blood/plasma	5	UV, 325 nm	133
Ronidazole + dimetridazole (and metabolites)	meat	2	UV, 320 nm	134
Benzimidazoles	milk	5	UV, 296 nm	3,138
Dapson (and metabolites)	milk	2–10	UV, 296 nm	96,139
Dapson + dimetridazole + sulphamethazine	meat	5–20	UV, 254, 296 and 315 nm	140,141
Clopidol	egg	10	UV, 270 nm	142
Trimethoprim	milk	5	UV, 289 nm	143
Oxytetracycline	blood/plasma	50	UV, 350 nm	144

[a] Flu. = fluorescence detection.

recoveries. Furthermore, the detection of a large number of drugs in general can only be achieved by using a rather non-selective detector such as UV-Vis in LC and TLC, and flame ionization detection (FID) in GC. This will result in relatively high limits of detection for some or even all of the drugs. The highly desirable use of more selective sample pretreatment, especially that of involving highly (immuno)affinity SPE materials will be discussed below.

In a multi-method designed for the determination of as many as 60 veterinary drugs in edible products, the above problems could essentially be solved by applying a multi-step sample clean-up followed by LC separation on up to three different LC systems (including at least one gradient) with UV-Vis diode-array detection, and one capillary GC separation which involved derivatization and electron-impact detection (ECD) [131,155,156]. With this procedure chloramphenicol and meticlorpindol could be determined at the sub-ppb level (<1 μg/kg) after clean-up on a miniaturized silica column and derivatization with BSA or hexamethyldisilane (HMDS)/trimethylchlorosilane (TMCS)/pyridine [156]. Fig. 6 shows the schematic of the procedure for extraction, separation and detection of veterinary drug residues

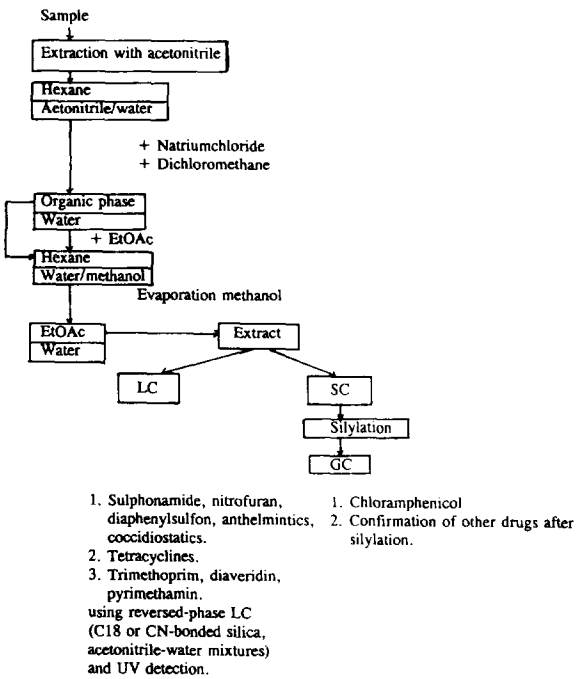

Fig. 6. Schematic of analytical procedures for determination of 60 veterinary drugs in edible products [131].

in milk, eggs and animal tissue. Even then, as was stressed by the author, there is a need for confirmation of positive findings [131].

Selectivity can also be introduced by (chemical) transformation of drug(s) of interest into compounds that possess favourable detection characteristics. In GC, (precolumn) derivatization with a reagent that enables trace-level determination with nitrogen–phosphorus detection (NPD) or ECD detectors for sensitive nitrogen–phosphorus, and halogen or other hetero-atom detection, respectively, is quite common [156,157]. With TLC, spraying of the plate with a suitable reagent prior to development is used for many drugs, e.g. sulphonamides [158], tranquillizers [159], nitrofurans [155,160] and tetracyclines [161]. With both GC and TLC, such derivatization procedures are performed off-line and are therefore not very attractive because the automation potential is low and side-products are often formed which may interfere in the determination of the analyte(s) of interest. The same is true for off-line and is true to a lesser extent (no automation problem)–for on-line pre-column LC derivatization. A review of pre-column (and post-column) conversions of drugs, which lead to the formation of fluorescent products is given in ref. [50].

Post-column LC reaction has a number of advantages [162]. For example, (i) the LC separation is not influenced, (ii) the procedure is on-line and can, therefore, easily be automated, and (iii) the reaction can be incomplete as long as it is reproducible (and the analyte conversion is sufficiently high to permit detection). However, there are also disadvantages because the LC eluent often is not the ideal reaction medium, some additional band broadening may be introduced, and an additional pulseless pump is (often) required for reagent introduction. The last aspect explains why photochemical and solid-state reactors are often preferred for post-column reaction detection.

Various post-column reactions for veterinary drug analysis have been reported [162–164]. They include reactions to transform the analytes into products that are highly fluorescent, UV-Vis absorbing or electrochemically active. The reaction principles include chemical derivatization, ion-pair formation, photoconversion and enzyme induction. Two interesting examples are shown in Figs. 7 and 8. In both instances, sample pretreatment involved on-line dialysis. Oxytetracycline [OTC; tetracycline (TC) used as internal standard] was determined in muscle, liver, milk, and egg using a polystyrene enrichment column, as a result of the addition of natriumhydroxide (NaOH) and irradiation at 366 nm resulting in highly fluorescent derivatives were formed (Fig. 7). The determination of a series of sulphonamides also involved trace enrichment on XAD-4 material and post-column derivatization with *p*-dimethylaminobenzaldehyde (DMAB) (Fig. 8). Other typical examples are given in Table 7.

Immunoaffinity-based sample treatment is a powerful tool for the selective clean-up of samples. In principle a high degree of enrichment and, consequently, low limits of detection can be obtained [79]. Secondly, the clean-up step con-

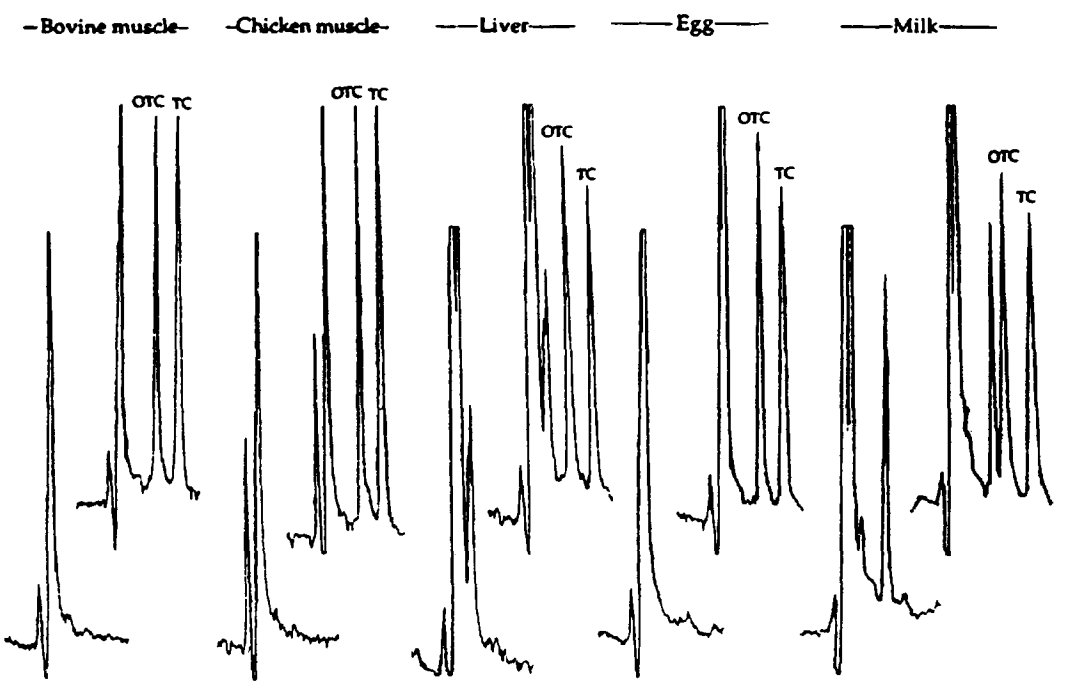

Fig. 7. LC-fluorescence detection of extracts of blanks (lower trace) and samples spiked with (oxy)tetracyclines (upper trace). Conditions: (ASTED system); LLE with hexane and eluent acetonitrile–0.02 *M* orthophosphoric acid/0.005 *M* heptanesulphonic acid (23:77, v/v); analytical column 5 μm PLRP-S (150 × 4.6 mm I.D.); irradiation at 366 nm (knitted reaction coil: 10 × 0.3 mm I.D.); excitation at 358 nm and emission at 460 nm. Spiking level: 50 ng/g OTC and 500 ng/g TC, except egg: 15 ng/g OTC and 150 ng/g TC in milk: 10 ng/ml OTC and 100 ng/ml TC. Range: 1 mV full scale, except milk: 2 mV full scale. Recording time: 13.5 min, except milk: 16 min. For dialysis and other conditions, see Ref. [78].

siderably adds to the selectivity of the procedure, which may even allow unambiguous analyte identification in combination with non-mass spectrometric confirmation techniques. If a mixture of antibodies raised against a (related group of) veterinary drugs is immobilized on a suitable stationary phase, which is then used to pack an enrichment cartridge, the simultaneous clean-up of one–or several–group(s) of drugs is possible. This would be very useful in multi-residue analysis. Immunoaffinity SPE can easily be combined with conventional analytical procedures for separation and detection. The approach has recently been reviewed by Van Ginkel [169] and Katz [170], and off-line and on-line applications have been reported for anabolics [171], β-agonists and a number of antibiotics [172–176]. Polyclonal antibodies immobilized on Sepharose have repeatedly been used for the selective sorption, and sample clean-up, of some β-agonists (e.g. clenbuterol and salbutamol) and corticosteroids (e.g. dexamethasone) in biological matrices [174,175]. The on-line extraction of diethylstilbestrol from urine has recently been performed by injecting samples directly onto an immunoaffinity column containing immobilized antidiethylstilbestrol antibodies bound to a Sepharose matrix combined with LC–continuous-flow (cf) fast-atom bombardment (FAB)-MS system [171]. Fig. 9 shows a set-up of on-line precolumn sample clean-up using immunoaffinity chromatography [175].

The use of mass spectrometric detection to increase the selectivity of multi-residue analyses will be discussed in the Section 3.2.3..

Capillary zone electrophoresis (CZE) is a technique which originally was mainly used for the separation of biological macromolecules.

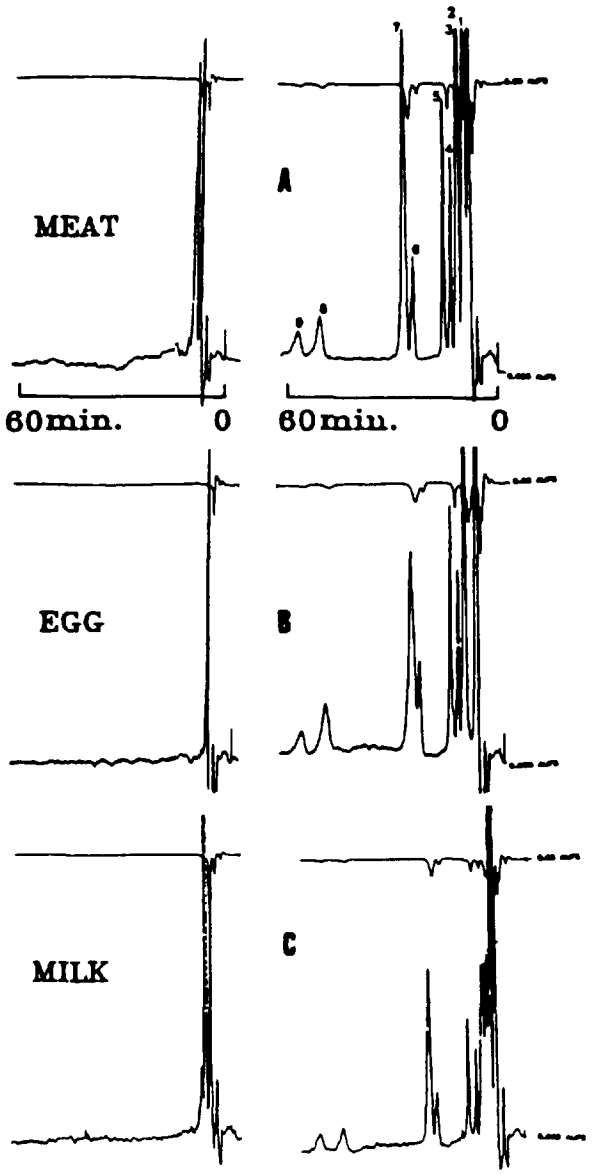

Fig. 8. LC–UV chromatograms of extracts of sulphonamides of blank and spiked meat (A, 100 μg/kg), egg (B, 50 μg/kg) and milk (C, 25 μg/l) samples. Conditions: enrichment column (60 × 4.6 mm I.D.) XAD-4; eluent acetonitrile–0.05 *M* sodium acetate (pH 4.6) (175:825, v/v); analytical column 10 μm LiChrosorb RP-8 (250 × 4.6 mm I.D.); derivatization with DMAB; detection at 450 nm, 0.005 AUFS. Peaks: 1 = sulphanilamide (SA), 2 = sulphathiazole (STh), 3 = sulphadiazine (SD), 4 = sulphamerazine (SM), 5 = sulphamethazine (SMZ), 6 = sulphadoxine (SDX), 7 = dapsone (DDS) + sulphatroxazole (STX) + sulphamethoxazole (SMX), 8 = sulphadimethoxine (SDM), 9 = sulphaquinoxaline (SQX). For further details on conditions, see Ref. [96].

However, its intrinsic high separation efficiency makes CZE an interesting alternative to chromatographic techniques for the separation of smaller molecules, especially when additional discrimination can be introduced as, e.g. with micellar electrokinetic chromatography [177]. With this technique drug monitoring in body fluids can elegantly be performed [178,179]. In addition, the introduction of chiral selectors such as cyclodextrins or α_1-acid glycoprotein enables the direct separation of drug enantiomers [180,181]. Because of the small, nl-range, injection volumes and the inherently low sensitivity of detection principles commonly used with this technique, its applicability in residue analysis is rather limited. However, if novel strategies which are being developed to increase injection volumes by means of on-line sample preconcentration turn out to be successful [182], this will enable the future use of CZE techniques in drug residue analysis.

3.2.2. Single-residue screening methods

The above discussion on multi-residue screening methods shows that, in relatively simply equipped laboratories, only microbiological methods can be used for routine application. However, to quote two examples, the extensively used antimicrobial chloramphenicol and the sulphonamides cannot be detected with sufficient sensitivity by using such methods [34,84]. This has prompted the development of simple specific tests for these compound classes, which should be used in addition to the microbiological methods suited for other microbials.

Immunoassays are widely used in therapeutic drug monitoring for humans [2]. The antibody–antigen interaction is highly selective and theoretically enables analytical procedures to be carried out without sample treatment. However, non-specific binding of matrix components that are present in large excess is a distinct problem [183]. In practice, therefore, some form of sample pretreatment is necessary. The assay is performed by bringing the antibodies into contact with the analyte and adding an amount of radio-, enzyme-, or fluorescent-labelled analyte, which competes with the non-labelled analyte for the

Table 7
Examples of post-column reaction detection of veterinary drugs using LC analyses

Drug	Matrix	Detection limit (μg/kg)	Detection method[a]	References
Carbadox (and metabolites)	egg/kidney/ liver/plasma	1	UV-Vis, 390–420 nm addition of 0.5 *M* NaOH	5 19
Nicarbazin	feed/egg	15	Vis., 440 nm, derivatization with 0.5 *M* NaOH	26
Flumequine	egg/meat/milk	5–10	Flu., ex. 240 nm, em. >370 nm reaction with sulphuric acid	38
Amprolium	egg/meat/feed	5	Flu., ex. 365 nm, em. >470 nm derivatization with hexacyanoferrate	40
Oxytetracycline	egg/milk/ muscle/liver	1 3–8	Flu., ex. 358 nm, em. 460 nm on-line addition of NaOH, irradiation at 366 nm	78
Sulphonamides	egg/meat/milk	25	Vis., 450 nm, derivatization with dimethylaminobenzaldehyde	96
Fenbendazole (and metabolites)	serum	25	Flu., ex. 300 nm, em. 342 nm on-line irradiation with mercury source, 254 nm	165
Ampicillin (and other thioethers)	urine/plasma	200	Amp., +0.4 V, oxidation by on-line generated bromine	166
Sulphaguanidine	egg	10	Vis., 450 nm, derivatization with dimethylaminobenzaldehyde	167
Aspoxicillin	broncho-alveolar	100	ECD, 800 mV, photolysis	168

[a] Amp. = amperometric detection.

available binding sites [183,184]. The amount of labelled analyte bound is then determined directly or after the addition of a suitable substrate that is transformed into a selectively detectable product. Nowadays, most immunochemical residue methods are enzyme-linked immunosorbent assays (ELISA), radio-immunoassays (RIA) or fluorescence polarisation immunoassays (FPIA).

A large number of ELISA-based methods for veterinary drug residues is commercially available as a kit [81]. For example, there are at least four kits on the market for the detection of the most extensively used sulphonamide, sulphadimidine, in urine, milk or plasma. Two of these are classical ELISA methods requiring microtiter plates and a spectroscopic reader [185,186]; the remaining two tests are in the form of a card or cup, and require no further instrumentation [187,188].

All these tests are intended for pre-slaughter or pre-milk delivery screening of animals and milk. The limits of detection range from 10 μg/l in milk to 100 μg/l in plasma and 400 μg/l in urine. The latter two limits are supposed to be sufficiently low to ensure that animals negative in the test will not contain violative levels (>100 μg/kg) of sulphadimidine in meat [189,190]. In all instances only minimal sample handling is required. A very sensitive (1 μg/l) direct competitive ELISA test for sulphadimidine in milk has been reported [191]. Most methods show up to 10% cross-reactivity of sulphamerazin, a closely related sulphonamide.

The second veterinary drug compound for

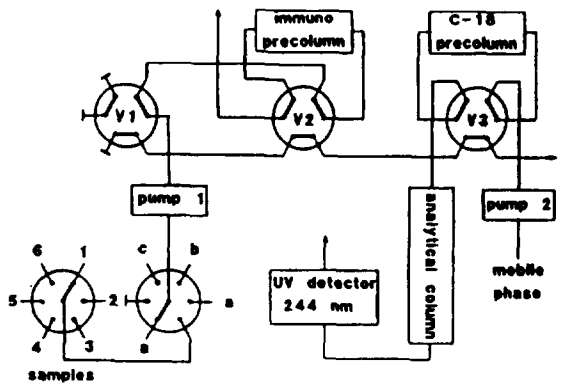

Fig. 9. Set-up of the on-line automated LC system using an immunoaffinity precolumn for the determination of clenbuterol residues in urine samples. Conditions: enrichment column Sepharose-immobilized polyclonal antibodies against clenbuterol, C_{18} precolumn (10 × 2 mm I.D.); analytical column 5 μm LiChrospher 60 RP-select B (125 × 4 mm I.D.); UV detection at 244 nm. Schedule of analytical procedure is presented in Ref. [175].

which several immunochemical tests have been developed is chloramphenicol. ELISA and RIA methods using both monoclonal and polyclonal antibodies have been described for the low-level (< 10 μg/kg) screening of urine, milk, meat and eggs [9]. A streptavidin–biotin-modified ELISA was developed for the analysis of swine muscle [192] and a routinely used RIA method was compared with an ELISA assay [85] and a conventional GC procedure [193]. The RIA method was shown to be highly sensitive with a LOD of about 200 ng/kg and rapid; for screening of meat, eggs, and milk samples, the RIA is highly specific. Because of specific antisera used no noticeable cross-reactivity has been observed. The results obtained by means of RIA were satisfactorily confirmed by GC–ECD carried out after silylation of chloramphenicol. A commercially available card test [97] has been collaboratively tested for meat [194] and urine [195], and is now routinely used in the Netherlands for the control of chloramphenicol in meat. A successful attempt has been made to raise an antibody against the glucuronide of chloramphenicol [196].

ELISA methods for the screening of biological samples have also been developed for the benzimidazoles benomyl and thiabendazole [197], the ionophoric coccidiostat monensin [198], the cephalosporin cephalexin [199], and the antibiotic colistin [200]. A direct (on-farm) enzymatic assay for β-lactams in milk is commercially available [81,201].

Generally, the requirement of a confirmatory method in case of positive findings and the fact that such tests are basically single-compound tests, limit the usefulness of immunochemical assays as screening methods in residue monitoring programmes.

The development of biological sensors as selective and simple instruments for the on-site control of drug residues in edible products is an interesting challenge for analytical chemists. So far, only one scientific study has been reported, viz. for the development of a penicillin sensor based on immobilized penicillinase [202].

3.2.3. Confirmation

If a screening test indicates the presence of a violative concentration of a drug residue, the next steps are to establish whether the amount of residue exceeds the MRL and to confirm the identity of the residue.

3.2.3.1. Non-spectrometric methods

The identity of antimicrobials found positive in a microbiological screening test is generally confirmed by means of high-voltage electrophoresis [203,204]. Although this approach combines efficient separation with microbiological detection using different microorganisms, unfortunately neither quantitative nor direct structural information is provided. The combination of liquid–liquid extraction, HPTLC and microbiological detection (bioautography) has been proposed as an alternative confirmation technique for antimicrobials [205,206]. For penicillins, false positive samples can be identified by repeating the test in the presence of a β-lactamase. If the inhibition zone disappears in the presence of the β-lactamase, this proves that a penicillin is present. However, many newer β-lactams are less sensitive towards inactivation by β-lactamase. Moreover, the repeatability and analyte detectability provided by the combined approach

are rather poor, the determination of ionophoric drugs being an exception [37,91]. Another indirect confirmation method which uses a receptor assay (Charm-test) has been discussed above.

For a growing number of antimicrobials, chromatographic–mainly LC–methods are being developed for quantitation and confirmation. Relevant examples are given in Table 8.

3.2.3.2. Spectrometric methods

Confirmation methods should preferably provide direct structural information which is usually obtained by means of one of two spectroscopic techniques, viz. DAD or MS detection. In addition, increasing attention is being paid to FT-IR and NMR detection. It seems appropriate to briefly discuss these two techniques first.

Table 8
Typical examples of quantitation and confirmation methods used for antimicrobials

Drug	Matrix	Detection limit (μg/kg)	Detection method[a]	References
Cephapirin (and metabolite)	milk/serum	10–50	LC-DAD, 200–360 nm	83
Aspoxillin	plasma	1300	Micellar electrokinetic chromatography	178
Cephalosporins	serum/urine	2000	Flu., ex. 385 nm, em. 485 nm after LC post-column addition of fluorescamine. UV, 262 nm Amp., direct or after post-column reaction with bromine	207
β-Lactams	standard	20000	Micellar electrokinetic solution chromatography	208
Enrofloxacin, ciprofloxacin	tissue/serum	2–4	LC–UV, 279 nm, LC–DAD, 230–360 nm	209
Spiramycin	meat	50	LC–UV, 231 nm	210
Lincomycin	kidney	50	Cap. GC–NPD, after C_{18}-SPE and LC clean-up; derivatization with BSTFA, 30 min; 70°C	211
Amprolium	egg/ tissue	10 1000	GC–NPD	212
Sulphonamides (and metabolite)	meat/egg/milk	10	Cap. GC–TID, after GPC clean-up and derivatization with diazomethane,	213
Chloramphenicol	meat/egg/milk	10	HPBTA	213
Penicillin G	milk	2	CS-LC–UV, 210 nm	214
Penicillin, cloxacillin	muscle/ liver/kidney	5 10	CS-LC–UV, 210 nm	215
Fluorquinolones, theophylline	plasma	200	LC–UV, 280 nm	216
Corticosteroids	plasma	3	LC, pre-column derivatization with CDB and C_{18}-SPE clean-up	217
Sulphamethazine, N^4-acetylsulphamethazine	meat	20	LC–DAD, 220–340 nm	218
Olaquindox	muscle/liver	20	LC–UV, 350 nm	219

[a] BSTFA = bis(trimethylsilyl)trifluoroacetamide; HPBTA = heptafluorobutyricanhydride; CDB = 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole.

Nowadays, FT-IR can be coupled on-line with capillary GC using either the lightpipe or the matrix-isolation approach. However, it is still mostly used as a stand-alone technique. That is, identification and quantification are achieved by fraction collection of eluting LC peaks or the scraping off of TLC spots. In practice, the coupled technique is used to identify hormonal substances in injection spots by examining peaks in the 1800–500 cm^{-1} wavenumber region. This can help to identify this class of drugs with a high degree of certainty [145,220,221]. The removal of highly interfering/absorbing solvents before FT-IR detection either by deposition or by coupling LC and FT-IR on-line via a suitable (nebulizer) interface, and the use of a microscope enhance the potential of the technique for trace-level analysis. The combined use of solid phase extraction and ^1H NMR detection has been reported for the purification and identification of drugs and their metabolites in biological fluids [222,223]. This procedure strongly enhances analyte detectability in NMR detection method and can be used for the determination of drug metabolites in biological samples without extensive pretreatment of the complex matrices.

Today, DAD is frequently used routinely for detection in LC; it mainly provides information on the basis of the spectral match of the UV spectrum recorded for the peak of interest and that of the analyte standard, and of the purity of the peak [9,83,131,147,155,156,218]. DAD can only be used successfully for confirmation purposes when the sample clean-up has efficiently removed interfering UV-Vis absorbing compounds. Depending on the specific instrumentation and the UV-Vis absorbance characteristics of the target compound, between 5 and 25 ng of analyte should be injected onto a LC system to obtain a suitable UV-Vis spectrum.

There is general agreement about the fact that MS detection provides more structural information at low analyte levels than any other analytical technique. The mass spectrum contains information regarding the mass of parent and fragment ions and their relative abundance [224,225]. Selectivity is best when full spectral scans can be acquired to compare sample and standard peaks. Since this type of operation causes a loss of sensitivity, in general selected-ion monitoring (SIM) is often used as a compromise [220,225,226]. The limited number of characteristic fragment ions, so-called diagnostic ions, that has to be scanned, depends on the ionization mode selected, the relative abundance of the ions, and conditions such as legislative and confirmation requirements. Several MS-based techniques can be said to be rather sensitive, with typical detection limits, in the SIM mode, in the picogram range.

Electron-impact (EI) ionization is the mode which is used most frequently. It produces a large number of fragment ions and thus provides much relevant structural information. However, fragmentation often results in the formation of many rather non-specific, low-molecular-mass fragments, which each have a low abundance [224,227], while the molecular ion, which is of course the most characteristic ion in the spectrum, is often only marginally present in the spectrum. Therefore several other ionization techniques have been developed which reduce fragmentation and increase the intensity of the molecular ion. Such soft techniques are, e.g. positive and negative chemical ionization (PCI and NCI, respectively), and FAB. They yield only a limited number of specific high-molecular-mass fragments [225,228–230]. In general, the sensitivity obtained with the soft ionization techniques is somewhat lower than that of EI. An exception to this rule is NCI-MS which allows the selective and sensitive detection of analytes that contain groups with electron-capturing properties, such as chloramphenicol [228,231], nicarbazin [232,233], and detomidine [234].

MS detection is generally combined with capillary GC separation, which means in many cases that the analytes of interest have to be derivatized before analysis. In such cases, the imprecision generated by the combined effect of a variable derivatization yield and fluctuations in the MS parameters necessitates the addition of an internal standard [131,155,156]. Ideally, the internal standard should show a behaviour as closely analogous to that of the analyte as is possible, while still allowing separate detection.

Spiking of the sample with a isotopically labelled internal standard (isotope dilution) meets these criteria [226,235,236]. Unfortunately, in most cases such isotopes are not commercially available. Other internal standards, mostly isomers or close analogues of the analyte of interest, are therefore frequently used [9,156,237,238]. Reliable quantitative information without derivatization of the analytes of interest has been obtained for, e.g. nitroimidazoles [227], and also levamisole [239], clenbuterol [175] and penicillin residues [240].

In practice, the confirmation procedure is often divided into two steps. Firstly, the sample is analysed by means of a well established LC method. The amount of analyte is quantified and the fraction of the LC eluate containing the analyte is collected. Next, this fraction is processed to make it suitable for direct injection into a mass spectrometer or for GC–MS. Advantages of this approach over direct GC–MS analysis are: (a) extra clean-up through LC separation; (b) reliable quantitative information; (c) tentative identification by means of e.g. DAD detection; (d) use of the expensive and relatively vulnerable mass spectrometer in a limited number of cases only, viz. when the MRL is exceeded. Fig. 10 shows GC–MS chromatograms concerning the confirmation of the presence of residues of methyltestosterone in kidney fat [241]. The remainder of the extract was derivatized with either mono(trimethylsilyl)trifluoroacetamide (MSTFA) which results in the formation of trimethylsilane(TMS)-enol ethers (Fig. 10A), or with ethoxine(EOX)-TMS which results in the formation of ethoxine-silyl ethers (Fig. 10B).

In the past few years, on-line LC–MS has become a rather popular technique. This is especially due to the improved performance of, e.g. the thermospray (TSP), particle beam (PB), and electrospray (ESP) interfaces, with LC–TSP-MS no doubt being the alternative most frequently used [242,243]. This is exemplified by the several applications described in Table 9. Fig. 11 shows LC–TSP-MS chromatograms which confirm the presence of desacetylcephapirin and cephapirin in bovine milk [83]. Multi-residue analysis with microbore LC–ESP-MS has the advantage that no post-column splitting of the eluent is required and all of the analyte will be transferred into the ESP interface [252]. Today, tandem mass spectrometry, i.e. MS–MS, is also being used for drug residue confirmation. Applications have been reported for the residue analysis of tetracyclines [253], sulphonamides [254], a third-generation fluoroquinolone, danofloxacin [247], β-lactams [252], betamethasone and clenbuterol [255], metoprolol [238], and clenbuterol [256]. Tandem MS approaches generally provide sub-ng/g detection limits, more or less independent of the biological matrix which is analysed. The sensitive and specific determination of danofloxacin and its residues in liver is achieved by monitoring the two daughter ions with tandem MS detection [247]. Fig. 12 shows the effect of collision-induced dissociation (CID) of the protonated danofloxacin molecule which results in the formation of the two daughter ions.

Recent developments in on-line coupled LC–GC, especially normal-phase LC–GC, open the possibility to achieve the highly selective and fully automated determination of analytes in, preferably, non-aqueous samples or sample extracts. The alternative of SPE–GC to combine the trace-enrichment of analytes from aqueous samples on-line with GC analysis has already been referred to above. Until now, most LC–GC and related applications are in the area of environmental and food analysis [125,257].

3.3. Validation of results

In official residue control, regulatory action can only be taken after unequivocal identification of contaminated products. The control system should also be able to effectively identify suspected samples in a large population. This implies that the analytical results should be accurate and precise, i.e. agree with the actual situation. This can only be achieved when (i) adequate analytical methods are available, and (ii) the work is carried out by trained personnel under quality assurance conditions.

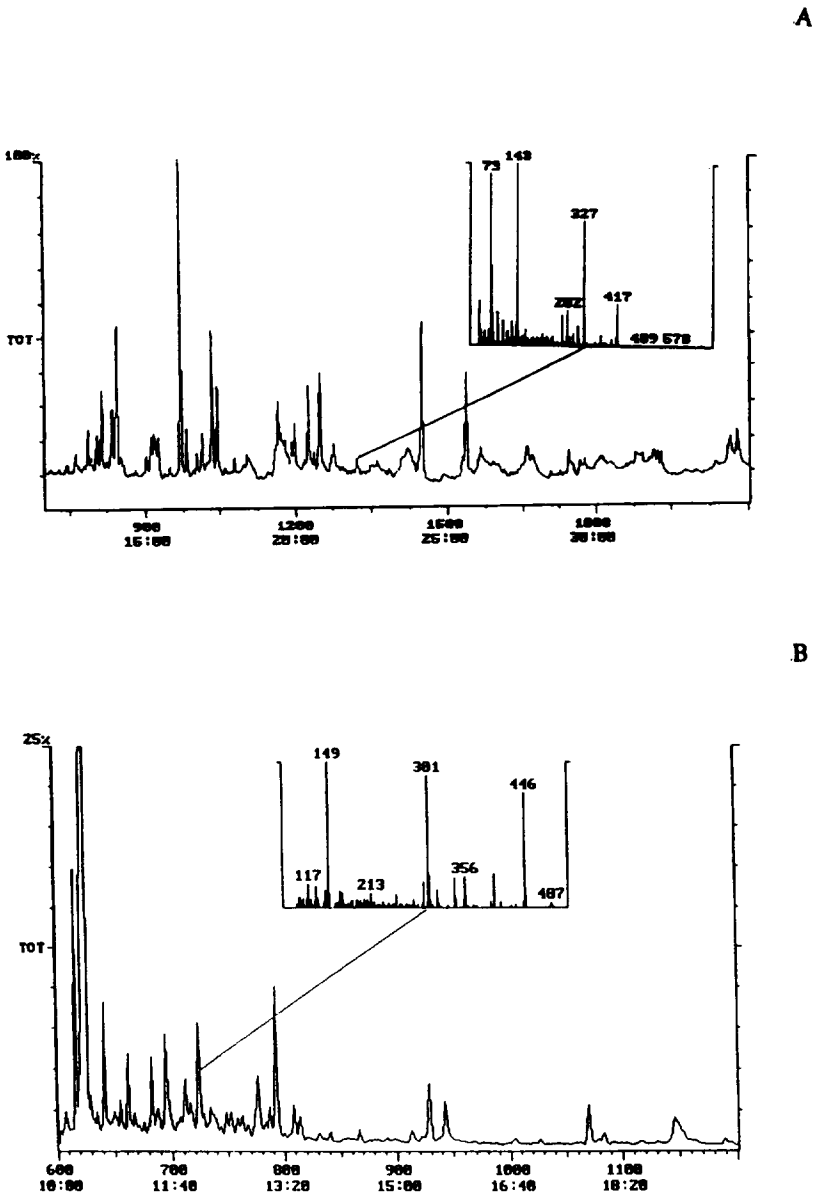

Fig. 10. GC–MS chromatograms confirming the presence of residues of methyltestosterone in a kidney fat extract, (A) after derivatization of extract with MSTFA resulting in formation of TMS-enol ethers, (B) after derivatization of extract with EOX-TMS resulting in formation of EOX-TMS ethers. For further experimental conditions, see Ref. [241].

3.3.1. Adequate analytical methods

Whether an analytical method is adequate or not depends upon the purpose for which it is going to be used. A screening method should allow the detection of all the suspected samples, using a relatively simple, routinely applicable procedure. A quantitative method should allow the user to reliably establish whether the residue level exceeds the MRL. Finally, a confirmatory method should give unequivocal evidence on the identity of the residue. Until recently an analytical method was believed to be adequate only

Table 9
Selected examples of MS-based confirmation procedures for veterinary drugs

Drug	Matrix	Detection limit (μg/kg)	Detection method[a]	References
Cephapirin, desacetylcephapirin	milk/serum	100–500	LC–TSP-MS, 4–5 ions	83
Diethylstilbestrol	urine	2	on-line LC–cf-FAB-MS	171
Nitroimidazoles	feed	100	GC–EI-MS, 5–6 ions, no derivatization	227
Chloramphenicol	egg/meat/ milk	0.03 5	GC–NCI-MS, methane, 5 ions, pyridine/TMCS/ HMDS disilylation	228
Sulphadimidine (and metabolites)	meat/organs	100	GC–PCI-MS, methane, 3 ions, diazomethane methylation	229
Lasalocid-Na	liver	400	GC–PCI-MS, isobutane, 4 ions, TMS silylation after collection of LC fraction	230
Chloramphenicol	urine/muscle egg	0.1	GC–NCI-MS, 4 ions BSTFA/TMCS silylation	231
Nicarbazin	tissues	20	LC–TSP-MS, 3 ions, C_{18} column, 20 μl injection	233
Detomidine	meat	0.2	GC–NCI-MS, methane, 2 ions	234
Levamisole	liver	5	GC–MS, 4 ions no derivatization	239
Sulphadimidine (and metabolites)	meat/organs	2–20	GC–EI-MS, 6–7 ions, diazomethane methylation after collection of LC fraction	244
Sulphonamides (and metabolites)	muscle	10–80	LC–TSP-MS	245
Furazolidone	muscle	0.6	LC–TSP-MS	246
Danofloxacin	liver	50	on-line LC–ESP-MS/MS	247
Chloramphenicol	plasma/milk tissue	2 1	LC–TSP-MS 2 ions	248
Pirlimycin	milk/liver	25	LC–TSP-MS, 4 ions	249
Oxolinic acid, nalidixic acid, piromicid acid	fish	10	LC–TSP-MS, 3 ions	250
Fenbendazole, oxfenbendazole	liver/ muscle	50 100	LC–TSP-MS	251

[a] BSTFA = bis(trimethylsilyl)trifluoroacetamide.

after it had successfully been tested in a full collaborative study. Because of the tremendous cost of such studies, the time necessary to fully complete a test, the rapid progress made in method development and the large number of compounds for which methods are required, this view has now changed. Within the EC, a group of experts has defined a number of criteria that have to be met by so-called reference methods for drug residue control [220,226]. These EC reference methods are to be used in case of an international dispute. The criteria include general demands on precision, limit of detection, limit of determination, accuracy, testing for interferences, calibration curves and the relationship between the established MRL and the limits of

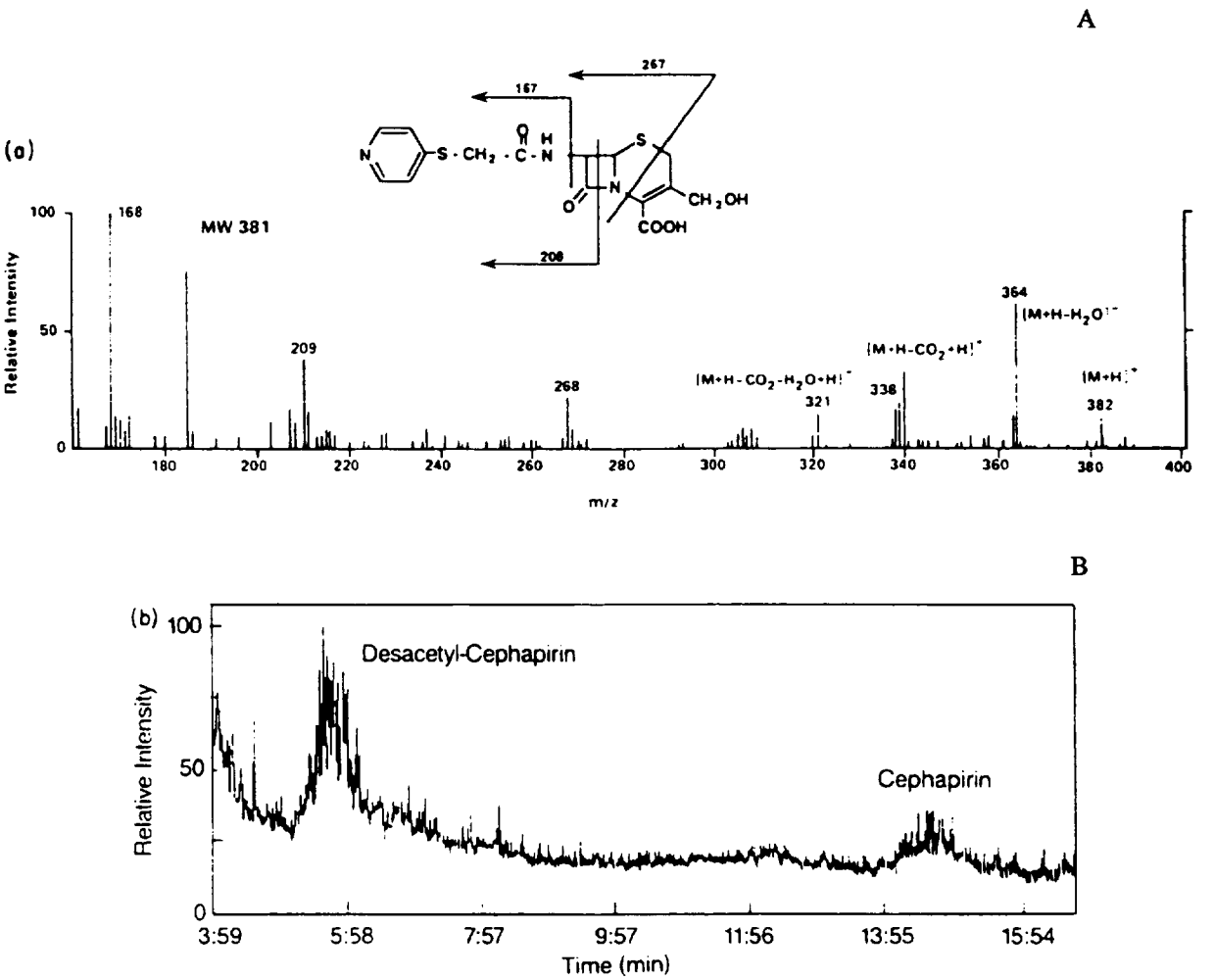

Fig. 11. LC–TSP-MS chromatograms (A) of desacetylcephapirin in dosed bovine milk (16 h after intramammary infusion), (B) of confirming the presence of desacetylcephapirin and cephapirin in bovine milk collected 16 h after intramammary infusion. Conditions: eluent isopropanol–30 mmol/l ammoniumacetate–acetic acid (6.5:93:0.5, v/v); analytical column Phenyl Spheri-5 (220 × 4.6 mm I.D.); positive-ion detection mode monitoring *m/z* 168, 268, 338, 364 and 382 for desacetylcephapirin and m/z 168, 209, 338 and 424 for cephapirin. For further experimental conditions, see Ref. [83].

identification and determination. Besides, a number of identification criteria for chromatographic (TLC, LC, GC), immunochemical, and spectrometric (MS, DAD, IR) techniques have been laid down. These criteria are very useful to establish whether a method has a sound basis and they provide guidelines for the analyst developing non-reference methods. With regard to international regulatory control, the "criteria approach" should result in a set of equivalent reference methods producing comparable results for each (group of) veterinary drug(s), rather than having one method laid down in detail. In 1992 the first version of a summarizing booklet describing the EC criteria and candidate reference methods has been issued by the commission of the EC [258].

One further aspect of interest should be mentioned here. The identification criteria for individual methods do not provide information on the degree of uncertainty left after application of the method. Apart from that, it would be very

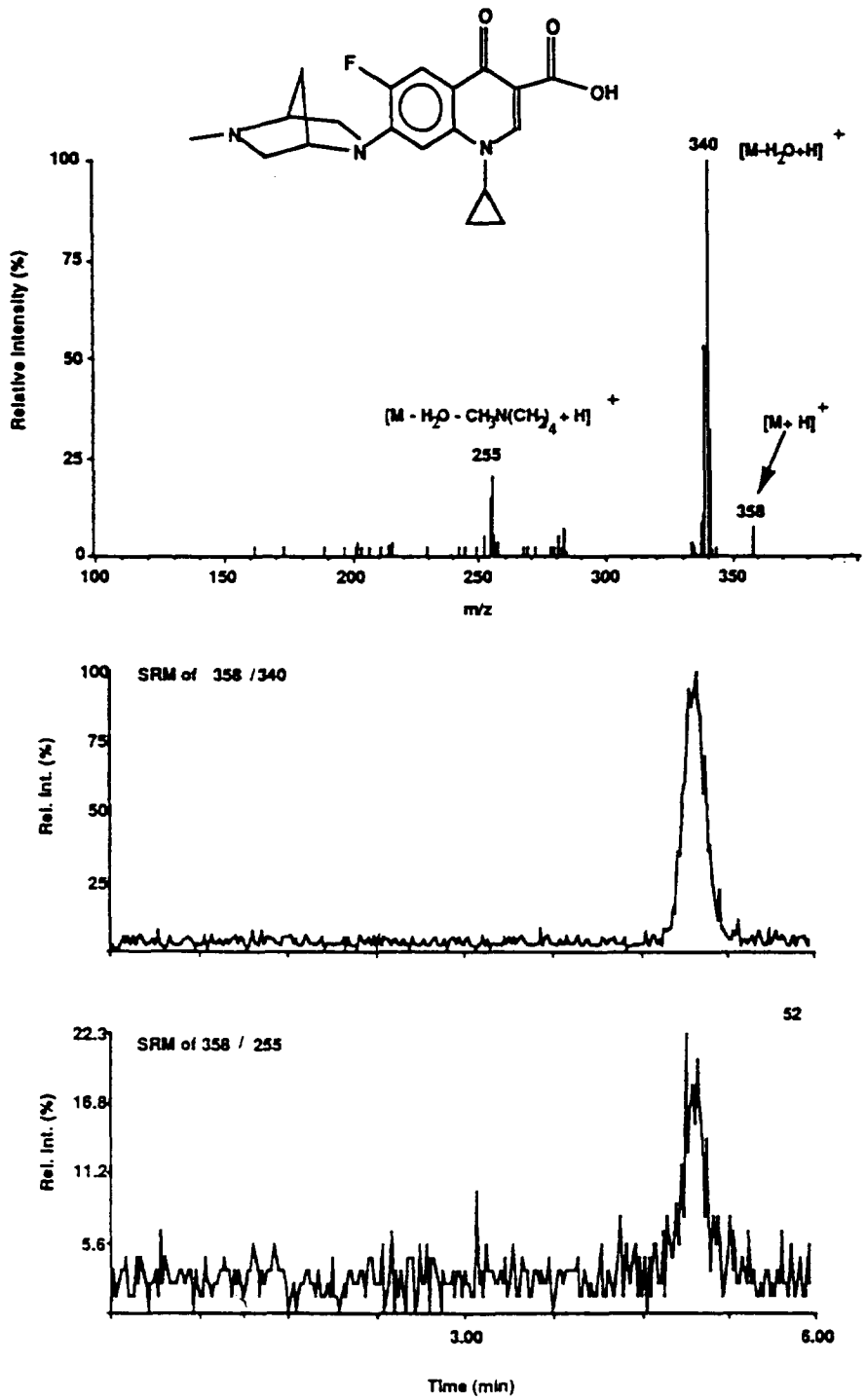

Fig. 12. LC–ES-MS–MS selected-reaction monitoring (SRM) chromatogram for an injection of 20 pg of danofloxacin and its collision-induced dissociation (CID) daughter spectrum. Conditions: Liquid–liquid extraction from cattle and chicken liver; eluent 0.1% acetonitrile–trifluoroacetic acid (20:80, v/v); analytical column Hypersil C_{18} (250 × 1 mm I.D.); SRM m/z 358 to 340 and m/z 358 to 255 [247].

helpful to have a means to quantify the certainty obtained when using combinations of methods. An attempt to establish chemometric criteria for the assessment of the certainty of qualitative analytical methods has been reported [9,145].

Establishing various types of criteria does not mean that there is no longer a need for collaborative studies. Apart from being the ultimate test for the quality of a method, collaborative studies are a very efficient way to familiarize laboratories with a method and, at a later stage, harmonize the quality of laboratories that are using it. Most importantly, however, when applying quantitative analytical methods, knowledge must be available on the maximum variation that can be expected when two laboratories independently analyse the same sample, i.e. the reproducibility of the method must be known. The reproducibility can only be established in a collaborative study. Last but not least, for all routine methods, whether qualitative or quantitative, their practicality is highly relevant. Such methods should not require very sophisticated or special (home-made) equipment and a trained technician should be able to readily use the method.

3.3.2. Quality assurance

Working under quality assurance conditions in essence means that there is a guarantee that the analytical method is carried out according to the procedure laid down, and that any deviations are registered and approved of by responsible staff. Actually, no statement is made regarding the quality of the method, but rather regarding the conditions under which it is carried out. Certification of analytical methods, or even laboratories, by (inter)national accrediting agencies will become increasingly important in the future European open-market situation. A method will only be certified if a number of internal or external quality assurance measures have been included in the procedure. A number of these are described in Ref. [9] quoted above, for the immunochemical, LC and GC–MS analysis of chloramphenicol residues in meat. Briefly, these measures comprise: (i) inclusion of recovery samples with each series; (ii) inclusion of blank samples; (iii) inclusion of known (internal) or "blind" (external) samples containing naturally incurred residues (control or reference material); (iv) prevention of contamination; (v) determination in duplicate; (vi) establishing criteria on the maximum allowable deviation of individual results from the mean recovery, the precision and the mean or certified value of a control/reference material (RM); (vii) involvement in quality-control collaborative studies.

With regard to the last aspect, it is clear that there is a need for (inter)national quality-control studies, even if one uses provisionally certified control materials containing one or more drugs. Such studies should aim at the comparison of results obtained by different laboratories using different methods [259]. In addition, certified reference materials (CRM) are needed such as those provided by United States Pharmacology (USP) and the EC, to test the accuracy of the total analytical methods. Biological reference materials still are very scarce and are currently only available for a number of hormones and chloramphenicol. Therefore other quality-control evaluation programmes have been initiated by individual countries to assess the quality of drug residue monitoring. One such programme is the Food Analysis Performance Scheme that has been set up by the United Kingdom Ministry of Agricultural and Fisheries and which evaluates samples containing oxytetracycline, sulphadimidine and chloramphenicol [260]. In summary, it is only the combination of high-quality analytical methods and laboratory quality assurance procedures, that can safeguard the quality of the food by identifying contaminated products and prevents false positive results.

4. Concluding remarks

The present review shows that many techniques are available and various strategies can be envisaged to determine residues of veterinary drugs in biological products. This leaves one with the problem of selecting the most appropriate control system for a specific situation.

In the Netherlands, there are peripheral meat

and milk control laboratories where, apart from one immunochemical card test, only microbiological assays are performed routinely. In addition, there are a few central laboratories which have facilities to perform a limited number of more sophisticated analytical methods. In this set-up it is desirable that cheap and simple screening techniques are developed for those drugs that have priority in order to utilize the available infrastructure as efficiently as is possible. In essence this means that the microbiological techniques still have a prominent position and research should be directed at broadening the scope of these assays. Priority drugs that can not be adequately monitored with the microbiological techniques should be monitored by means of receptor- or immuno-assays, preferably in a kit format. It is essential that receptor and antibody reagents are made commercially available to ensure a continuous and constant-quality supply. The receptor- and immuno-assays already require some extra expertise in the peripheral laboratories.

The screening of drugs not detectable with the simple bioassays has to take place in the central laboratories using physicochemical or more complex immunochemical multi-methods, which preferably should be automated and provide quantitative and structural information. Here, it seems better to use a number of tailor-made group-selective methods than one comprehensive method which will always cause compromising between the range of drugs that can be included and the achievable selectivity/sensitivity.

It may be attractive to use GC–MS or LC–MS methods for screening because of their good selectivity and–when working in the SIM mode–high sensitivity, although these techniques require a sophisticated laboratory environment. Therefore, in practice this approach is not often chosen for the screening of veterinary drugs. Still, in a number of countries multi-residue GC–MS methods have been developed and are currently in use for the control of banned anabolic hormones and β-agonists. The rather similar physicochemical properties of these analytes allow this approach. If, on the other hand, different ionization modes have to be used for various analytes, such as is the case with clenbuterol (PCI) and chloramphenicol (EI or NCI), then a separate mass spectrometer will be required for each individual routine analysis, which will make the approach much less interesting.

Samples found to be positive during screening have to be subjected to quantitation and/or confirmation analyses by means of more sophisticated methods at central laboratories. For antimicrobials, but possibly also for other drugs, high-efficiency electrophoretic techniques such as isotachophoresis and capillary zone electrophoresis, may become the methods of choice in the future; this will certainly be true if the analyte detectability can be further improved [182]. For other classes of drugs the combination of LC–DAD followed by off-line MS or GC–MS, and LC–MS analysis today are the methods of choice for quantitation and confirmation purposes. On-line combinations such as LC–DAD-TSP(or ESP)-MS, with dialysis and/or SPE for sample clean-up and/or trace enrichment, and on-line LC–LC-based systems which allow heartcutting, are attractive options for further research.

Generally speaking, it is beneficial to have a non-mass spectrometric screening/quantitation method, even if this would require a somewhat more laborious sample clean-up. The mass spectrometer can then be kept for identification of samples containing residues above the MRL or for those cases in which the analyte can not be

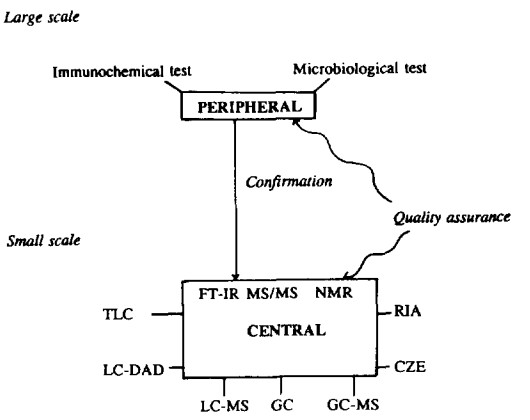

Fig. 13. Set-up of a residue control system based on a routine peripheral screening of anti-microbials and priority drugs, in combination with group-selective screening of other drugs and general confirmation, at central laboratories. The list of techniques should be considered typical rather than exhaustive.

detected selectively with any of the other techniques. Fig. 13 shows a schematic presentation of the set-up of a control system as discussed above.

Finally, the introduction of a residue control programme for a specific drug or group of drugs will have an impact on the use of these drug(s). The ability of the control programme to detect residues of a certain drug in edible products will discourage its use. If, on the other hand, it is known that residues of certain drugs can not (sensitively) be detected by the control system, non-obeyance of withdrawal periods will go unpunished and extra-label use of the drugs will become more attractive. A good example is the use of dapson with lactating cows. In 1984, in the Netherlands about 9% of the milk samples tested were found to contain residues of this sulphone drug which could not be detected by the penicillin-sensitive microbiological test. Introduction of an automated LC monitoring procedure (the Netherlands)–with which the high residue occurrence was detected–by the Food Inspection Service of Utrecht, followed by the introduction of a microbiological "sulpha test" in 1987 at the milk-control agencies resulted in a steady decrease in residue incidence to a level below 0.2% in 1988.

Appendix

Table A1
Gross composition of egg, milk and muscle

Component	Egg yolk	Egg white	Milk	Muscle
Water (%)	49	88	87	75
pH	6.0–6.8	7.6–9.4	6.7	5.7
Proteins (%)	16.5 lipovitellins phosvitins livetins lipoproteins avidin	10.5 ovalbumin ovotransferrin ovomucoid lysozyme ovomucin	3.3 casein (78%) lactalbumins lactoglobulins immunoglobulins lactoferrin	19.0 myofibrillar sarcoplasmic collagen elastin
Lipids (%)	33 triglycerides (66%) phospholipids (28%) cholesterol (5%)	0.03 – – –	3.7 triglycerides (95%) phospholipids (1%) cholesterol (0.3%)	2.5 neutral fat fatty acids phospholipids
Carbohydrates (%)	0.2 mainly glucose	0.5 –	4.7 mainly lactose	1.2 lactic acid
Enzymes	amylase esterase phosphatase catalase peptidase		peroxidase fosfatase lipase xantin-oxidase	aldolase cr.kinase other glycol. enzymes
Other	carotenoid pigments: xanthophylls zeaxantine, luthein		vitamins: A, D, E, K, B mineral salts (0.7%) (mainly calcium and potassium phosphates) inhibitors: agglutinins, peroxidase, lysozyme	vitamins mineral salts (0.7%) creatine

Table A2
Established maximum residue levels (MRLs) within the European Union

Compound	MRL (μg/g)	Matrix[a]	Species[b]	Remarks
Enrofloxacin	0.03	M, L, K	B, P, Ch	
Ivermectin	0.10	L	B	H_2B_{1a} metabolite
	0.04	F	B	
	0.015	L	O	
	0.02	F	P, Eq	
Closantel	1.00	M, L	B	
	3.00	K, F	B	
	1.50	M, L	O	
	5.00	K	O	
	2.00	F	O	
Triclabendazole	0.15	M, L, K	B, O	Sum of residues oxidizable to keto-triclabendazole
	0.05	F	B, O	
Flubendazole	0.50	L	Ch, G	
	0.20	M	Ch, G	
	0.40	E	Ch, G	
	0.01	M, L, K, F	P	
Oxibendazole	0.10	M, L, K, F	B, O, P, Eq	
	0.05	Mi	B, O	
Albendazole	0.10	M, F, Mi	B, O	Albendazole metabolites measured as 2-amino-benzimidazole-sulphone
	0.50	K	B, O	
	1.00	L	B, O	
Thiabendazole	0.10	M, L, K, F, Mi	B, O, Ca	Thiabendazole + 5-hydroxy metabolite
Febantel	1.00	L	n.s.	Sum of oxfendazole, oxfendazole-sulphone and fenbendazole
Fenbendazole Oxfendazole	0.01	M, K, F, Mi	n.s.	
Amitraz	0.05	M	P	Amitraz and metabolites measured as 2,4-dimethylaniline
	0.20	K,L	P	
Furazolidone	0.005	M, L, K, F	All	Intact 5-nitro structure Provisional to 06-1995
Other nitrofurans				No MRL can be fixed
Chloramphenicol	0.01	M, L, K, F	n.s.	No MRL for eggs and milk
Thiamphenicol	0.04	M, L, K, F	B, Ch	
Sulphonamides	0.10	M, Mi, L, K, F	n.s.	
Trimethoprim	0.05	M, Mi, L, K, F	n.s.	

Table A2 (*Continued*)

Compound	MRL (μg/g)	Matrix[a]	Species[b]	Remarks
Cefquinome	0.20	K	B	
	0.1	L	B	
	0.05	M, F	B	
Ampicillin	0.05	M, L, K, F	n.s.	
	0.004	Mi	B, O	
Amoxicillin	0.05	M, L, K, F	n.s.	
	0.004	Mi	B, O	
Benzylpenicillin	0.05	M, L, K, F	n.s.	
	0.004	Mi	B, O	
Cloxacillin	0.30	M, K, L, F	n.s.	
	0.03	Mi	B, O	
Oxacillin	0.30	M, K, L, F	n.s.	
	0.03	Mi	B, O	
Oxytetracycline	0.60	K	n.s.	
	0.30	L	n.s.	
	0.20	E	n.s.	
	0.10	M, Mi	n.s.	
	0.01	F	n.s.	
Levamisole	0.01	M, K, L, F, Mi	n.s.	
Azaperone	0.10	K	n.s.	Azaperol
	0.05	M, L, F	n.s.	
Carazolol	0.05	L, K	n.s.	
	0.005	M, F	n.s.	

[a] M = Muscle; Mi = milk; L = liver; K = kidney; E = eggs; F = fat.
[b] B = Bovine; O = ovine; P = porcine; Ch = poultry; Ca = caprine; Eq = equine; G = gamebirds; n.s. = not significant.

References

[1] M. Wenk, S. Vozeh and F. Follath, *Clin. Pharmacokin.*, 9 (1984) 475.
[2] J. Chamberlain, *Analysis of Drugs in Biological Fluids*, CRC Press, Boca Raton, FL, USA, 1985.
[3] W. van Leeuwen, H.W. van Gend and R. Kommerij, Report of the Food Inspection Service, IR/73/01/86/D13, Utrecht, Netherlands, 1986.
[4] K. Küng, J.L. Riond and M. Wanner, *J. Vet. Pharmacol. Therap.*, 16 (1993) 462.
[5] M.M.L. Aerts, W.M.J. Beek, H.J. Keukens and U.A.Th. Brinkman, *J. Chromatogr.*, 456 (1988) 105.
[6] J.M. Dewolney, L. Maes, J.P. Raynaud, F. Blanc, J.P. Scheid, T. Jackson, S. Lens and C. Verschueren, *Food Chem. Toxicol.*, 29 (1991) 477.
[7] J.F.M. Nouws, T.B. Vree, M.M.L. Aerts and J. Grondel, in W.A. Moats (Editor), *Agricultural Uses of Antibiotics*, ACS Symposium Series, Washington, D.C., USA, 1986, pp. 168–182.
[8] M.M.L. Aerts, I.M. Egberink, C.A. Kan, H.J. Keukens and W.M.J. Beek, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 46.
[9] H.J. Keukens, M.M.L. Aerts, W.A. Traag, J.F.M. Nouws, W.G. de Ruig, W.M.J. Beek and J.M.P. den Hartog, *J. Assoc. Off. Anal. Chem.*, 75 (1992) 245.

[10] L.H.M. Vroomen, Ph. D. Thesis, Wageningen, Netherlands, 1987.
[11] M. Petz, *Arch. Lebensmittelhyg.*, 35 (1984) 51.
[12] R.L. Epstein, V. Randecker, P. Corrrao, J.T. Keeton and H.R. Cross, *J. Agric. Food Chem.*, 36 (1988) 1009.
[13] J.J. O'Brien, N. Campbell and T. Conahan, *J. Hyg. Camb.*, 87 (1981) 511.
[14] M.C. Hermínia, M.E. Soares, J.O. Fernandes, M.L. Bastos and M. Ferreira, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 246–250.
[15] N. Haagsma, Ph. D. Thesis, Utrecht, Netherlands, 1988.
[16] S.Y. Hsu and R.L. Epstein, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 387–390.
[17] I. Steffenak, V. Hormazabal and M. Yndestad, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 646–649.
[18] A. Koole, L.A. Smit and N. Haagsma, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 415–418.
[19] G.M. Binnendijk, M.M.L. Aerts, H.J. Keukens and U.A.Th. Brinkman, *J. Chromatogr.*, 541 (1991) 401.
[20] N. Haagsma, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 41–49.
[21] J.J.H.M. Lohman, Ph. D. Thesis, Leiden, Netherlands, 1986.
[22] G. Ziv and F.G. Sulman, *Antimicrob. Agents Chemother.*, 2 (1977) 206.
[23] A. Rogstad, O.F. Ellingsen and C. Syvertsen, *Aquaculture*, 110 (1993) 207.
[24] M.D. Apley and D.W. Upson, *Am. J. Vet. Res.*, 54 (1993) 937.
[25] P. Walstra M.C van der Haven, Course on Dairy Technology, Agricultural University of Wageningen, Wageningen, Netherlands. 1977.
[26] M.M.L. Aerts, unpublished results.
[27] G. Ziv and F. Rasmussen, *J. Dairy Sci.*, 58 (1975) 938.
[28] R.A. Lawrie (Editor), *Meat Science*, Pergamon Press, Oxford, UK, 1979.
[29] M.M.L. Aerts, W.M.J. Beek and U.A.Th. Brinkman, *J. Chromatogr.*, 500 (1990) 453.
[30] V. Burgat-Sacaze, A.G. Rico and J.C. Panniset, in A.G. Rico (Editor), *Drug Residues in Animals*, Academic Press, Orlando, FL, USA, 1986.
[31] N.E. Weber, in J. Brunton and M.A. Mehlman (Editors), *Drug Residues in Food-producing Animals*, Pathotox Publishers, Park Forest South, USA.
[32] A.Y.H. Lu, G.T. Miwa and P.G. Wislocki, *Rev. Biochem. Toxicol.*, 9 (1988) 1.
[33] R.M. Parker and I.C. Shaw, *Analyst*, 113 (1988) 1875.
[34] J.F.M. Nouws, *Arch. Lebensmittelhyg.*, 32 (1981) 103.
[35] M. Bennion (Editor), *The Science of Food*, Harper and Row, San Francisco, CA, USA, pp. 383–406.
[36] F.S. Shenstone, in T.C. Carter (Editor), *Egg Quality; A Study of the Hen's Egg*, Oliver and Boyd, Edinburgh, UK, 1968.
[37] E. Kolsters, C.A. Kan and M.M.L. Aerts, Determination of ionophoric antibiotics in eggs, depletion studies with laying hens (in Dutch), RIKILT, Wageningen, Netherlands, 1989.
[38] W. van Leeuwen and H.W van Gend, Food Inspection Service Report IR/73/03/89/D33, Utrecht, Netherlands, 1989.
[39] M. Petz, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 528–532.
[40] W. van Leeuwen H.W. van Gend, *Z. Lebensm. Unters. Forsch.*, 186 (1988) 500.
[41] M. Vertommen, Gezondheids Dienst voor Pluimvee, Doorn, Netherlands, Personal Communication.
[42] M.F. Geertsma, J.F.M. Nouws, J.L. Grondel, M.M.L. Aerts, T.B. Vree and C.A. Kan, *Vet. Quarterly*, 9 (1987) 67.
[43] C.A. Kan, H.W. van Gend and M.M.L. Aerts, *World Poultry*, 7 (1991) 51.
[44] M. Petz, *Z. Lebensm. Unters. Forsch.*, 180 (1985) 267.
[45] J. Wang, *Intern. Lab.*, October (1985) 68.
[46] V. Stará and M. Kopanica, *Anal. Chim. Acta*, 186 (1986) 21.
[47] A.C. Bratton, E.K. Marshall, D. Babitt and A.R. Hendrickson, *J. Biol. Chem.*, 128 (1939) 537.
[48] F. Tishler, J.L. Sutter, J.N. Bathish and H.E. Hagman, *J. Agric. Food Chem.*, 16 (1968) 50.
[49] B. Shaikh and W.A. Moats, *J. Chromatogr.*, 643 (1993) 369.
[50] M. Petz, in *Analytik von Rückstände Pharmakologisch Wirksame Stoffe*, B. Behr's Verlag, Hamburg, Germany, 1988, pp. 34–61 and 73–123.
[51] W.A. Moats, in W.A. Moats (Editor), *Agricultural Uses of Antibiotics, ACS Symp. Series*, Washington DC, USA, pp. 154–168.
[52] E.H. Allen, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 990.
[53] B. Shaikh and E.H. Allen, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 1007.
[54] D. Weiss and A. McDonald, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 971.
[55] J. Breiter, R. Helger and H. Lang, *Forensic Sci.*, 7 (1976) 131.
[56] S. Chulavatnatol and B.G. Charles, *J. Chromatogr.*, 615 (1993) 91.
[57] C.M. Moore, *J. Forensic Sci. Soc.*, 30 (1990) 123.
[58] R.W. Frei and K. Zech (Editors), *Selective Sample Handling and Detection in HPLC, Vol A*, Elsevier, Amsterdam, Netherlands, 1988.

[59] A. Farjam, G.J. de Jong, R.W. Frei, U.A.Th. Brinkman, W. Haasnoot, A.R.M. Hamers, R. Schilt and F.A. Huf, *J. Chromatogr.*, 452 (1988) 419.
[60] M.W.F. Nielen, Ph. D. Thesis, Free University, Amsterdam, Netherlands, 1988.
[61] M. Krogh, A.S. Christophersen and K.E. Rasmussen, *J. Chromatogr.*, 621 (1993) 41.
[62] M.C. Rouan, *J. Chromatogr.*, 340 (1985) 361.
[63] G.M. Binnendijk, W.M.J. Beek and M.M.L. Aerts, Report 90.07, RIKILT, Wageningen, Netherlands, 1990.
[64] P.B. Kruger, C.F. de V. Albrecht and P.P. van Jaarsveld, *J. Chromatogr.*, 612 (1993) 191.
[65] P. Campíns-Falcó, R. Herráez-Hernández and A. Sevillano-Cabeza, *J. Chromatogr.*, 619 (1993) 177.
[66] J.V. Poszluszny and R. Weinberger, *Anal. Chem.*, 60 (1988) 1953.
[67] I.H. Hagestam and P.C. Pinkerton, *Anal. Chem.*, 57 (1985) 1757.
[68] H. Takahagi, K. Inoque and M. Horiguchi, *J. Chromatogr.*, 352 (1986) 369.
[69] E. Reh, *J. Chromatogr.*, 433 (1988) 119.
[70] D.J. Anderson, *Anal. Chem.*, 65 (1993) 434R.
[71] B.G. Österdahl, H. Johnsson and I. Nordlander, *J. Chromatogr.*, 337 (1985) 151.
[72] J.D. Weber and M.D. Smedley, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 445.
[73] R. Chiou, R.J. Stubbs and W.F. Bayne, *J. Chromatogr.*, 416 (1987) 196.
[74] D. Mourot, M. Dagorn and D. Delepine, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 810.
[75] J.-C. Jordan and B.M. Ludwig, *J. Chromatogr.*, 362 (1986) 263.
[76] J. Unruh, E. Piotrowski, D.P. Schwartz and R. Barford, *J. Chromatogr.*, 519 (1990) 179.
[77] H.W. van Gend and E.M. Mattern, *de Ware(n) Chemicus*, 13 (1983) 83.
[78] T. Agasøster, *Food Addit. Contam.*, 9 (1992) 615.
[79] C. van de Water, D. Tebbal and N. Haagsma, *J. Chromatogr.*, 478 (1989) 205.
[80] E. Schneider, E. Usleber, R. Dietrich, E. Märtlbauer and G. Terplan, in N. Haagsma, A. Ruiter, P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 627–631.
[81] E. Usleber, E. Märtlbauer, E. Schneider and R. Dietrich, *Archiv. Lebensmittelhyg.*, 45 (1994) 32.
[82] K.L. Tyczkowska, K.L. Anderson and A.L. Aronson, *J. Chromatogr.*, 614 (1993) 123.
[83] K.L. Tyczkowska, R.D. Voyksner and A.L Aronson, *J. Vet. Pharmacol. Therap.*, 14 (1991) 51.
[84] J.F.M. Nouws, N.J.G. Broex, J.M.P. den Hartog and F. Driessen, *Arch. Lebensmittelhyg.*, 39 (1988) 135.
[85] M. Beck, E. Märtlbauer and G. Terplan, *Arch. Lebensmittelhyg.*, 38 (1987) 93.
[86] R.V. Winchester, *New Zealand J. Sci.* 21 (1987) 553.
[87] A.M. Marti, A.E. Mooser and H. Koch, *J. Chromatogr.*, 498 (1990) 145.
[88] M.S. Gentleman, H.M. Burt, D.D. Kitts and K.M. McErlane, *J. Chromatogr.*, 633 (1993) 105.
[89] D.J. Moore, P.J. Perrino, C.P. Klerer and P. Robertson, *J. Chromatogr.*, 612 (1993) 310.
[90] M. Janecek, M.A. Quilliam, M.R. Bailey and D.H. North, *J. Chromatogr.*, 619 (1993) 63.
[91] M.M.L. Aerts, Ph. D. Thesis, Free University, Amsterdam, Netherlands, 1990.
[92] M.D. Osselton, *J. Forensic Sci. Soc.*, 17 (1977) 189.
[93] G. de Groot, B.C.A. Tepas and G. Storm, *J. Pharm. Biomed. Anal.*, 6 (1988) 927.
[94] A.L. Lehninger, *Biochemistry*, Worth Publishers, New York, NY, USA, pp. 157–181.
[95] B. Roudaut, *Br. Poult. Sci.*, 30 (1989) 265.
[96] M.M.L. Aerts, W.M.J. Beek and U.A.Th. Brinkman, *J. Chromatogr.*, 435 (1988) 97.
[97] G. Schelhaas, *Fortschr. Veter. Med.*, 20 (1974) 272.
[98] D. Guggisberg, A.E. Mooser and H. Koch, *J. Chromatogr.*, 624 (1992) 425.
[99] A.S. Barker and C.C. Walker, *J. Chromatogr.*, 624 (1992) 105.
[100] S.E. Katz, in W.A. Moats (Editor), *Agricultural Uses of Antibiotics, ACS Symp. Series*, Washington DC, USA, pp. 142–154.
[101] G.P. Dimenna, F.S. Lyon, F.M. Thompson, J.A. Creegan and G.J Wright, *J. Agric. Food Chem.*, 37 (1989) 668.
[102] J.R. Bishop and C.H. White, *J. Food Prot.*, 47 (1984) 647.
[103] W. Heeschen and G. Suhren, *Milchwissenschaft*, 41 (1986) 749.
[104] J. van der Stroom, Ph. D. Thesis (in Dutch), Utrecht, Netherlands, 1985.
[105] F.J. Müller, *Archiv. Lebensmittelhyg.*, 44 (1993) 105.
[106] L.A. Oudenkirk, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 985.
[107] A.E.M. Vermunt, J. Stadhouders, G.J.M. Loeffen and R. Bakker, *Neth. Milk Dairy J.*, 47 (1993) 31.
[108] H.W. van Gend, Annual Report, Food Inspection Service, Utrecht, Netherlands, 1988.
[109] E.H. Seymour, G.M. Jones and M.L. McGilliard, *J.Dairy Sci.*, 71 (1988) 539.
[110] G.M. Jones and E.H. Seymour, *J. Dairy Sci.*, 71 (1988) 1691.
[111] Food and Drug Administration, *Udder Topics*, 16 (1993) 3.
[112] M. van Schothorst and J.F.M. Nouws, in D. Strauch (Editor), *Animal Production and Environmental Health*, Elsevier, Amsterdam, Netherlands, 1987, pp. 265–279.
[113] L. de Zutter, K. Koenen-Dierick and J. van Hoof, *Tijdschr. Dierengeneesk.*, 54 (1985) 445.
[114] J.F.M. Nouws, N.J.G. Broex, J.M.P. den Hartog, F. Driessens and W.D.M. Driessen-van Lankveld, *Tijdschr. Diergeneeskd.*, 113 (1988) 247.
[115] N.J.G. Broex, J.M.P. de Hartog and J.F.M. Nouws, *Tijdschr. Diergeneeskd.*, 113 (1988) 254.

[116] F.K. Stekelenburg, *Tijdschr. Diergeneeskd.*, 118 (1993) 293.
[117] G. Anhalt, S. Wenzel and P. Conrad, *Arch. Lebensmittelhyg.*, 27 (1976) 201.
[118] M. Yoshida, D. Kubota, S. Yonezawa, H. Nogawa, H. Yoshimura and O. Ito, *Jpn. Poult. Sci.*, 13 (1976) 129.
[119] S.E. Charm and R. Chi, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 304.
[120] A. Carlsson and L. Björck, *Milchwissenschaft*, 44 (1989) 7.
[121] G. Suhren and W. Heeschen, *Dtsch. Molkerei-Ztg.*, 48 (1987) 1566.
[122] E. Zomer, B. Salter, D. Legg, J. Lawton Scheemaker, L. Plumley and S.E. Charm, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 706–709.
[123] W.A. Moats, K.L. Anderson, J.E. Rushing and D.P. Wesen, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 495–498.
[124] G. Audunsson, *Anal. Chem.*, 60 (1988) 1340.
[125] J.J. Vreuls, G.J. de Jong, R.T. Ghijsen and U.A.Th. Brinkman, *J. Assoc. Off. Anal. Chem.*, 77 (1994) 306.
[126] M.W.F. Nielen, A.J. Valk, R.W. Frei, U.A.Th. Brinkman, Ph. Mussche, R. de Nijs, B. Ooms and W. Smink, *J. Chromatogr.*, 393 (1987) 69.
[127] J.C. Pearce, J.A. Kelly, K.A. Fernandes, W.J. Leavens and R.D. McDowall, *J. Chromatogr.*, 353 (1986) 371.
[128] M.C. Rouan, J. Campestrini, J.B. Lecaillon, J.P. Dubois, M. Lamontagne and B. Pichon, *J. Chromatogr.*, 456 (1988) 45.
[129] Equipment available from, e.g., Gilson; Spark; Varian; Waters Assoc.
[130] R.D. McDowall, J.C. Pearce and G.S. Markitt, *J. Pharm. Biomed. Anal.*, 4 (1986) 3.
[131] R. Malisch, *Z. Lebensm. Unters. Forsch.*, 182 (1986) 385.
[132] A.T. Andresen and K.E. Rasmussen, *J. Liq. Chromatogr.*, 13 (1990) 4051.
[133] T. Agasøster and K.E. Rasmussen, *J. Chromatogr.*, 564 (1991) 171.
[134] H.J. Keukens, M.C. Elema, W.M.J. Beek and A. Boekestein, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 410–414.
[135] E. Staal, M.J.B. Mengelers and M.M.L. Aerts, The application of on-line GPC in the determination of a number of antibiotics in animal tissues, RIKILT, Wageningen, Netherlands, 1989.
[136] R.A. Williams, R. Macrae and M.J. Shepherd, *J. Chromatogr.*, 477 (1989) 315.
[137] M.J.B. Mengelers, A. Polman, M.M.L. Aerts, H.A. Kuipers and A.S.J.A.M. van Miert, *J. Liq. Chromatogr.*, 16 (1993) 257.
[138] W.M.J. Beek and M.M.L. Aerts, Report 86.98, RIKILT, Wageningen, Netherlands, 1986.
[139] M.B. Brinkman, H.W. van Gend and E.M. Mattern, *Z. Lebensm. Unters. Forsch.*, 183 (1986) 97.
[140] E.M. Mattern and H.W. van Gend, Food Inspection Service Report IR/73/06/87/D23, Utrecht, Netherlands, 1987.
[141] E.M. Mattern and H.W. van Gend, Food Inspection Service Report IR/73/05/87/D22, Utrecht, Netherlands, 1987.
[142] E.M. Mattern, H.W. van Gend and C.A. Kan, *Z. Lebensm. Unters. Forsch.*, 190 (1990) 25.
[143] R.L.J. Goverde and H.W. van Gend, Food Inspection Service Report IR/73/04/88/D29, Utrecht, Netherlands, 1988.
[144] T. Agasøster and K.E. Rasmussen, *J. Chromatogr.*, 570 (1991) 99.
[145] W.G. de Ruig, G. Dijkstra and R.W. Stephany, *Anal. Chim. Acta*, 223 (1989) 277.
[146] J.J. Laurensen and J.F.M. Nouws, *J. Chromatogr.*, 472 (1989) 321.
[147] W.M.J. Beek and M.M.L. Aerts, *Z. Lebensm. Unters. Forsch.*, 180 (1985) 211.
[148] H.J. Keukens and M.M.L. Aerts, *J. Chromatogr.*, 464 (1989) 149.
[149] T.B. Waggoner and M.C. Bowman, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 813.
[150] M. Horie, K. Saito, Y. Hoshino, N. Nose, E. Mochizuki and H. Nakazawa, *J. Chromatogr.*, 402 (1987) 301.
[151] W. van Leeuwen and H.W. van Gend, Food Inspection Service Report IR/73/03/89/D33, Utrecht, Netherlands, 1989.
[152] F. Lombardi, R. Ardemagni, V. Colzani and M. Visconti, *J. Chromatogr.*, 576 (1992) 129.
[153] M. Petz, *J. Chromatogr.*, 423 (1987) 217.
[154] H. Irth, Ph. D. Thesis, Free University, Amsterdam, Netherlands, 1989.
[155] R. Malisch, *Z. Lebensm. Unters. Forsch.*, 183 (1986) 253.
[156] R. Malisch, *Z. Lebensm. Unters. Forsch.*, 184 (1987) 467.
[157] D.R. Knapp, *Handbook of Analytical Derivatizations*, Wiley Interscience, New York, NY, USA, 1978.
[158] N. Haagsma, B. Dieleman and B.G.M. Gortemaker, *Vet. Quarterly*, 6 (1984) 8.
[159] N. Haagsma, E.R. Bathelt and J.W. Engelsma, *J. Chromatogr.*, 436 (1988) 73.
[160] M. Petz, *Lebensmittelchem. Gerichtl. Chem.*, 39 (1985) 16.
[161] Y. Ikai, H. Oka, N. Kawaniwa, M. Yamada, K. Harada and M. Suzuki, *J. Chromatogr.*, 411 (1987) 313.
[162] J.F. Lawrence, U.A.Th. Brinkman and R.W. Frei, in J.S. Krull (Editor), *Reaction Detection in Liquid Chromatography*, Marcel Dekker, New York, NY, USA, 1986.
[163] U.A.Th. Brinkman, *Chromatographia*, 24 (1987) 190.
[164] C. de Ruiter, Ph. D. Thesis, Free University, Amsterdam, Netherlands, 1989.

[165] M. Uihlein and E. Schwab, *Chromatographia*, 15 (1982) 140.
[166] W.Th. Kok, J.J. Halvax, W.H. Voogt, U.A.Th. Brinkman and R.W. Frei, *Anal. Chem.*, 57 (1985) 2580.
[167] M.M.L. Aerts, W.M.J. Beek, C.A. Kan and J.F.M. Nouws, *Arch. Lebenmittelhyg.*, 37 (1986) 142 and idem erratum 38 (1987) 27.
[168] T. Yamazaki, T. Ishikawa, H. Nakai, M. Miyai, T. Tsubota and K. Asona, *J. Chromatogr.*, 615 (1993) 180.
[169] L.A. van Ginkel, *J. Chromatogr.*, 564 (1991 363.
[170] S.E. Katz and M. Siewierski, *J. Chromatogr.*, 624 (1992) 389.
[171] E. Davoli, R. Fanelli and R. Bagnati, *Anal. Chem.*, 65 (1993) 2679.
[172] W. Haasnoot, S.M. Ezkerro and H.J. Keukens, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 347–351.
[173] W. Takasaki, M. Asami, S. Muramatsu, R. Hayashi, Y. Tanaka, K. Kawabata and K. Hoshiyama, *J. Chromatogr.*, 613 (1993) 67.
[174] S.M.R. Stanley, B.S. Wilhelmi and J.P. Rodgers, *J. Chromatogr.*, 620 (1993) 250.
[175] W. Haasnoot, M.P. Ploum, R.J.A. Paulussen, R. Schilt and F.A. Huf, *J. Chromatogr.*, 519 (1990) 323.
[176] A. Farjam, N.V. van de Merbel, A.A. Nieman, H. Lingeman and U.A.Th. Brinkman, *J. Chromatogr.*, 589 (1992) 141.
[177] P.H. Corran and N. Sutcliffe, *J. Chromatogr.*, 636 (1993) 87.
[178] H. Nishi, T. Fukuyama and M. Matsuo, *J. Chromatogr.*, 515 (1993) 245.
[179] W. Thormann, S. Lienhard and P. Wernly, *J. Chromatogr.*, 636 (1993) 137.
[180] T.E. Peterson, *J. Chromatogr.*, 630 (1993) 353.
[181] S. Li and D.K. Lloyd, *Anal. Chem.*, 65 (1993) 3684.
[182] M.E. Swartz and M. Merion, *J. Chromatogr.*, 632 (1993) 209.
[183] D. Arnold, in *Analytik von Rückstände Pharmakologisch Wirksame Stoffe*, B. Behr's Verlag, Hamburg, Germany, 1988, pp. 61–76 and pp. 185–201.
[184] C. Blake and B.J. Gould, *Analyst*, 109 (1984) 533.
[185] P. Singh, B.P. Ram and N. Sharkov, *J. Agric. Food Chem.*, 37 (1989) 109; commercially available in Europe through NOVO Food Diagnostics, Copenhagen, Denmark.
[186] RANSULR-test, developed by J.McCaughey, Dept. Agriculture, Belfast, N.I., UK; commercially available through Randox Laboratories, Crumlin, Co. Antrim, N.I., UK.
[187] CITE sulphamethazine test kit, Agritech, Portland, USA; commercially available in Europe through IDEXX, Mainz, FRG.
[188] La CarteR test kit, Environmental Diagnostics, Burlington, USA; commercially available in Netherlands by Transia-Biocontrol, Waddinxveen.
[189] R.D. Ashworth, R.L. Epstein, M.H. Thomas and L.T. Frobish, *Am. J. Vet. Res.*, 47 (1986) 2596.
[190] V.W. Randecker, J.A. Reagan, R.E. Engel, D.L. Soderberg and J.E. McNeal, *J. Food Protect.*, 50 (1987) 115.
[191] D.E. Dixon-Holland and S.E. Katz, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 447.
[192] C. van de Water and N. Haagsma, *J. Assoc. Off. Anal. Chem.*, 73 (1990) 534.
[193] D. Arnold and A. Somogyi, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 984.
[194] H.J. Keukens, J.M.P. den Hartog and M.M.L. Aerts, Collaborative study of the determination of chloramphenicol in meat using the La Carte immunochemical test kit, in preparation.
[195] L.A. van Ginkel and H.J. van Rossum, RIVM Report 388702-004, Bilthoven, Netherlands, 1989.
[196] C. Hock and F. Lieman, *Arch. Lebensmittelhyg.*, 36 (1985) 125.
[197] W.H. Newsome and P.G. Collins, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 1025.
[198] M.E. Mount and D.L. Failla, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 201.
[199] T. Kitagawa, Y. Gotoh, K. Uchihara, Y. Kohri, T. Kinoue, K. Fujiwara and W. Ohtani, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 915.
[200] T. Kitagawa, W. Ohtani, Y. Maeno, K. Fujiwara and Y. Kimura, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 661.
[201] P. Jaksch, *Dtsch. Molkerei-Ztg.*, 29 (1988) 898; commercially available through Smith Kline animal health products, West Chester, USA.
[202] T.C. Yerian, G.D. Christian and J. Ruzicka, *Anal. Chem.*, 60 (1988) 1256.
[203] K. Koenen-Dierick, L. de Zutter and J. van Hoof, *Arch. Lebensmittelhyg.*, 38 (1987) 128.
[204] G.N. Frerichs and M.D. Chandler, *J. Biol. Stand.*, 10 (1982) 205.
[205] E. Neidert, P.W. Saschenbrecker and F. Tittiger, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 197.
[206] C.D.C. Salisbury, C.E. Rigby and W. Chan, *J. Agric. Food Chem.*, 37 (1989) 105.
[207] M.D. Blanchin, W.Th. Kok and H. Fabre, *Chromatographia*, 24 (1987) 625.
[208] H. Nishi, N. Tsumagari, T. Kakimoto and S. Terabe, *J. Chromatogr.*, 477 (1989) 259.
[209] K. Tyczkowska, K.H. Hedeen, D. Aucoin and A.L. Aronson, *J. Chromatogr.*, 493 (1989) 337.
[210] T. Nagata and M. Saeki, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 644.
[211] W.A. Farrington, S.D. Cass, A.L. Patey and G. Shearer, *Food Addit. Contam.*, 5 (1988) 67.
[212] M. Petz, H.-P. Thier and H. Vogt, *Z. Lebensm. Unters. Forsch.*, 170 (1980) 329.
[213] H. von Holtmannspötter and H.-P. Thier, *Deutsche Lebensmittel-Rundschau*, 10 (1982) 347.
[214] W.A. Moats, *J. Chromatogr.*, 507 (1990) 177.
[215] W.A. Moats, *J. Chromatogr.*, 593 (1992) 15.
[216] J.D. Davis, L. Aarons and J.B. Houston, *J. Chromatogr.*, 621 (1993) 105.
[217] M. Katayama, Y. Masuda and H. Taniguchi, *J. Chromatogr.*, 612 (1993) 33.

[218] M. Horie, K. Saito, Y. Hoshino, N. Nose N. Hamada and H. Nakazawa, *J. Chromatogr.*, 502 (1990) 371.
[219] T. Nagata and M. Saeki, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 706.
[220] W.G. de Ruig, R.W. Stephany and G. Dijkstra, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 487.
[221] W.G. de Ruig and J.M. Weseman, *J. Chemom.*, 4 (1990) 61.
[222] I.D. Wilson and J.K. Nicholson, *J. Pharm. Biomed. Anal.*, 6 (1989) 151.
[223] I.D. Wilson, J.K. Nicholson, F.Y.K. Ghauri, C.A. Blackledge, *Anal. Proc. (London)*, 28 (1992) 217.
[224] G.M. Pesyna, F.W. McLafferty, R. Venkataraghevan and H.E. Dayringer, *Anal. Chem.*, 47 (1992) 352.
[225] J.A. Sphon, *J. Assoc. Off. Anal. Chem.*, 61 (1978) 1247.
[226] EC-Directive 89/610/EC, Commission of the European Community, 32 (1989) L-351.
[227] W.J. Morris, G.J. Nandrea, J.E. Roybal, R.K. Munns, W. Shimoda and H.R. Skinner jr, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 630.
[228] P. Fürst, C. Krüger, H.A. Meemken and W. Groebel, *Dtsch. Lebensm. Rundsch.*, 84 (1988) 108.
[229] J.E. Matusik, C.G. Guyer, J.N. Geleta and C.J. Barnes, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 546.
[230] D.R. Newkirk and C.J. Barnes, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 581.
[231] E. van der Heeft, A.P.J.M. de Jong, L.A. van Ginkel, H.J. van Rossum and G. Zomer, *Biol. Mass Spectr.*, 20 (1991) 763.
[232] G.S.F. Bories, J.C. Peleran J.M. Wal, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1521.
[233] J.J. Lewis, T.D. Macy and D.A. Garteiz, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 577.
[234] L. Vuorilehto, J.S. Salonen and M. Antilla, *J. Chromatogr.*, 530 (1990) 137.
[235] W.A. Garland, B.J. Hobson, G. Chen, G. Weiss, N.R. Felicito and A. MacDonald, *J. Agric. Food Chem.*, 28 (1990) 273.
[236] F.B. Suhre, R.M. Simpson and J.W. Shafer, *J. Agric. Food. Chem.*, 29 (1981) 727.
[237] P. Fürst, in *Analytik von Rückstände Pharmakologisch Wirksame Stoffe*, B. Behr's Verlag, Hamburg, Germany, 1988, pp. 159–188.
[238] A. Walhagen, L.E. Edholm, C.E.M Heeremans, R.A.M. van der Hoeven, W.M.A Niessen, U.R. Tjaden and J. van der Greef, *J. Chromatogr.*, 474 (1989) 257.
[239] S. Porter, R. Patel, S. Neate and P. Osso, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 548–552.
[240] G. Langeloh and M. Petz, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 433–437.
[241] H.F. de Brabander, L. Hendriks, F. Smets, P. Delahaut, P. Batjoens, L. Leyssens and G. Pottie, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 211–216.
[242] W.M.A. Niessen and J. van der Greef (Editors), *Liquid Chromatography–Mass Spectrometry; Principles and Applications, Chromatographic Science Series 58*, Marcel Dekker, New York, NY, USA, 1992.
[243] A.L. Burlingame, T.A. Baille and D.H. Russell, *Anal. Chem.*, 64 (1992) 467R.
[244] G.D. Paulson, A.D. Mitchell and R.G. Zaylskie, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 1000.
[245] G. Balizs, L. Benesch-Girke and S.A. Hewitt, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 155–159.
[246] W.J. Blanchflower, R.J. McCracken and D.G. Kennedy, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 201–205.
[247] R.P. Schneider, J.F. Ericson, M.J. Lynch and H.G. Fouda, *Biol. Mass Spectr.*, 22 (1993) 595.
[248] W.J. Blanchflower, A. Cannavan, R.J. McCracken, S.A. Hewitt and D.G. Kennedy, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 196–200.
[249] R.E. Hornish and A.R. Cazers, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 382–386.
[250] M. Horie, K. Saito, N. Nose, M. Tera and H. Nakazawa, *J. Liq. Chromatogr.*, 16 (1993) 1463.
[251] W.J. Blanchflower, A. Cannavan and D.G. Kennedy, *Analyst*, 119 (1994) 1325.
[252] R.F. Straub and R.D. Voyksner, *J. Chromatogr.*, 647 (1993) 167.
[253] P. Traldi, S. Daolio, B. Pelli, R. Maffei-Facino and M. Carini, *Biomed. Mass. Spectr.*, 12 (1985) 493.
[254] W.C. Brumley, Z. Min, J.E. Matusik, J.A.G. Roach, C.J. Barnes, J.A. Sphon and T. Fazio, *Anal. Chem.*, 55 (1983) 1405.
[255] J. Henion, G.A. Maylin and B.A. Thomson, *J. Chromatogr.*, 271 (1983) 107.
[256] J. van der Greef, C.J.M. Arts, M. van Baak and E.R. Verheij, in N. Haagsma, A Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 35–38.
[257] K. Grob, in W. Bertsch, W.G. Jennings and P. Sandra (Editors), *On-line coupled LC-GC*, Hüthig, Heidelberg, Germany, 1991.
[258] R.J. Heitzman (Editor), EC Report EUR 14126 ISBN 92-826-4095-7, 345 pages, 1993.
[259] L.A. van Ginkel, R.W. Stephany, H.J. van Rossum and M. Bos, in N. Haagsma, A. Ruiter and P.B. Czedik-Eysenberg (Editors), *Proc. EuroResidue II Conf., Veldhoven, Netherlands*, 1993, pp. 308–312.
[260] FAPAS secretariat, CSL Food Science Laboratory, Norwich, UK.