The potential of monoclonal antibodies against ampicillin for the preparation of a multi-immunoaffinity chromatography for penicillins†

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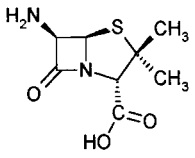
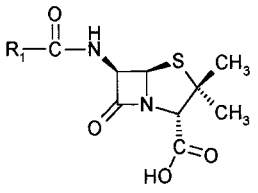
Monoclonal antibodies (Mab) against ampicillin were prepared by immunization of mice with an ampicillin–keyhole limpet hemocyanin conjugate coupled by a glutaraldehyde method. Sensitivity and specificity of these antibodies were tested in a direct competitive enzyme immunoassay, in which an ampicillin–horseradish peroxidase conjugate prepared by a carbodiimide method served as the labelled antigen. According to their cross-reactivities with the other β-lactam antibiotics, the Mabs could be divided into two groups, which are represented by the clones designated 1D1 and 3B5. While Mab 3B5 (IgG_1) showed no major cross-reactions with the other penicillins frequently used in veterinary medicine except for amoxicillin (108%), Mab 1D1 (IgG_{2a}) had marked cross-reactivities with most of the 17 tested β-lactam antibiotics (*e.g.*, amoxicillin 187%, penicillin G 31%, cloxacillin 30%, dicloxacillin 44%, and oxacillin 14%). The detection limits for ampicillin, calculated from the antibiotic concentration giving 30% binding inhibition, were 11.7 (Mab 3B5) and 16.6 ng ml^{-1} (Mab 1D1). To prepare multi-immunoaffinity chromatography columns, Mab 1D1 and a previously described antibody against cloxacillin (Mab 1F7) were each coupled to CNBr activated sepharose. The capacity of the resulting immunosorbents was approximately 6.6 and 5.4 μg ml^{-1} gel for ampicillin and cloxacillin, respectively. Recoveries of amoxicillin, ampicillin, cloxacillin, dicloxacillin, penicillin G and oxacillin (in buffer solutions) from the produced immunoaffinity columns were in the range from 67 to 100%.

Introduction

Among the antimicrobial drugs used in veterinary medicine, β-lactam antibiotics, and in particular penicillins (see Fig. 1 and 2), are most frequently used for the prophylaxis and therapy of infectious diseases, thus implying the risk of residues in milk and animal tissues. In order to protect consumers from risks correlated with drug residues in milk and meat products, the European Union has laid down[1] maximum residue limits (MRL) for amoxicillin, ampicillin, penicillin G, penethamate (milk: 4 μg kg^{-1}; animal tissues: 50 μg kg^{-1}), isoxazolyl penicillins (milk: 30 μg kg^{-1}; animal tissues: 300 μg kg^{-1}) and nafcilline (milk, animal tissues: 30 μg kg^{-1}).



6-Aminopenicillanic acid (6-APA)



Penicillin nucleus

Fig. 1 Structure of 6-aminopenicillanic acid (6-APA) and general structure of penicillins containing the 6-APA nucleus. For side chain residues see Fig. 2.

The most common screening methods for penicillin residues in food of animal origin are microbial inhibition tests and receptor type assays, which either lack specificity or are too limited for the group-specific detection. Physico-chemical methods, however, are both expensive and time-consuming and thus restricted to confirmatory analyses.[2,3] Carson *et al.*[4] compared several determinative HPLC and GC methods for the analysis of β-lactam antibiotics in milk and stated that currently none of the tested procedures fulfils the demands of a suitable, multi-residue analysis method. Particularly prolonged, tedious and laborious extraction steps and interferences in the chromatograms hampered the analyses. During the last decade immunoaffinity (IA) chromatography had proved its worth as a versatile tool for the efficient isolation of contaminants and residues from food and feed. [5–7] For the analysis of penicillins, however, this promising approach in analytical chemistry currently plays no role mainly due to the lack of appropriate antibodies. The only described approach for the preparation of a multi-analyte penicillin IA column failed due to the instability of the employed egg yolk antibodies.[8] Typically, antibodies for penicillins described so far are either highly specific[9] or have limited group specificity for a few closely related compounds.[10–14] Recently, a polyclonal antiserum with strong cross-reactions with all penicillins containing the intact 6-aminopenicillanic acid (6-APA) nucleus has been described.[15] However, the limited availability of polyclonal antibodies limits their use for IA chromatography. This paper describes the production and characterization of monoclonal antibodies against ampicillin with broad group-specificity towards pen-

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icillins, and their applicability for the preparation of IA columns.

Experimental

Chemicals, buffers and equipment

Amoxicillin, ampicillin, azlocillin, bacampicillin, carbenicillin, cephalothin, cephalexin, cloxacillin, dicloxacillin, epicillin, metampicillin, methicillin, moxalactam, oxacillin, penicillin G, penicillin V, pheneticillin, piperacillin, ticarcillin, and 6-APA (free acids or as sodium/potassium salts) were obtained from Sigma-Aldrich Vertriebs GmbH, Deisenhofen, Germany. Horseradish peroxidase (HRP), glutaraldehyde 25% solution (v/v), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDPC), and 3,3′,5,5′-tetramethylbenzidine (TMB) were also from Sigma. Fetal calf serum (FCS) and cell culture medium (Dulbeccos modified Eagles medium, DMEM) were purchased from Biochrom (Berlin, Germany). Rabbit anti-mouse immunoglobulins were obtained from DAKO Diagnostica (Hamburg, Germany). Keyhole limpet hemocyanin (KLH), polyethylene glycol 4000 and the Mouse Hybridoma Subtyping Kit were from Boehringer, Mannheim, Germany. Monoclonal antibodies against cloxacillin (Mab 1F7)[10] and enzyme immunoassays (EIA) for penicillins[15] and cloxacillin[10] were used as described earlier (Table 1).

Stock solutions (1 mg ml^{-1}) of ampicillin, amoxicillin, epicillin, methicillin, and 6-APA were prepared in distilled water: stock solutions of all other antibiotics were prepared in methanol. Antibiotic standard solutions for EIA and IA chromatography were prepared in phosphate buffered saline (PBS; 0.01 mol l^{-1}, pH 7.3; phosphate buffer containing 0.1 mol l^{-1} NaCl). Stock solutions and standard solutions were prepared daily. Hydrolyzed penicillins were prepared by adding sodium hydroxide (1 mol l^{-1}; 10 μl) to 1 ml of stock solution and incubating at room temperature for 30 min.

The dilution buffer for coating microtitre plates (Maxisorb; Nunc, Wiesbaden, Germany) with anti-mouse immunoglobulins was carbonate–bicarbonate buffer (0.05 mol l^{-1}; pH 9.6; 100 μl per well). To block free protein binding sites of the plates, PBS containing sodium caseinate (30 g l^{-1}) was used (200 μl per well). The microtitre plate wash solution was distilled water containing 8.5 g l^{-1} NaCl and 0.25 ml l^{-1} Tween 20. Enzyme conjugate dilution buffer was PBS containing 10 g

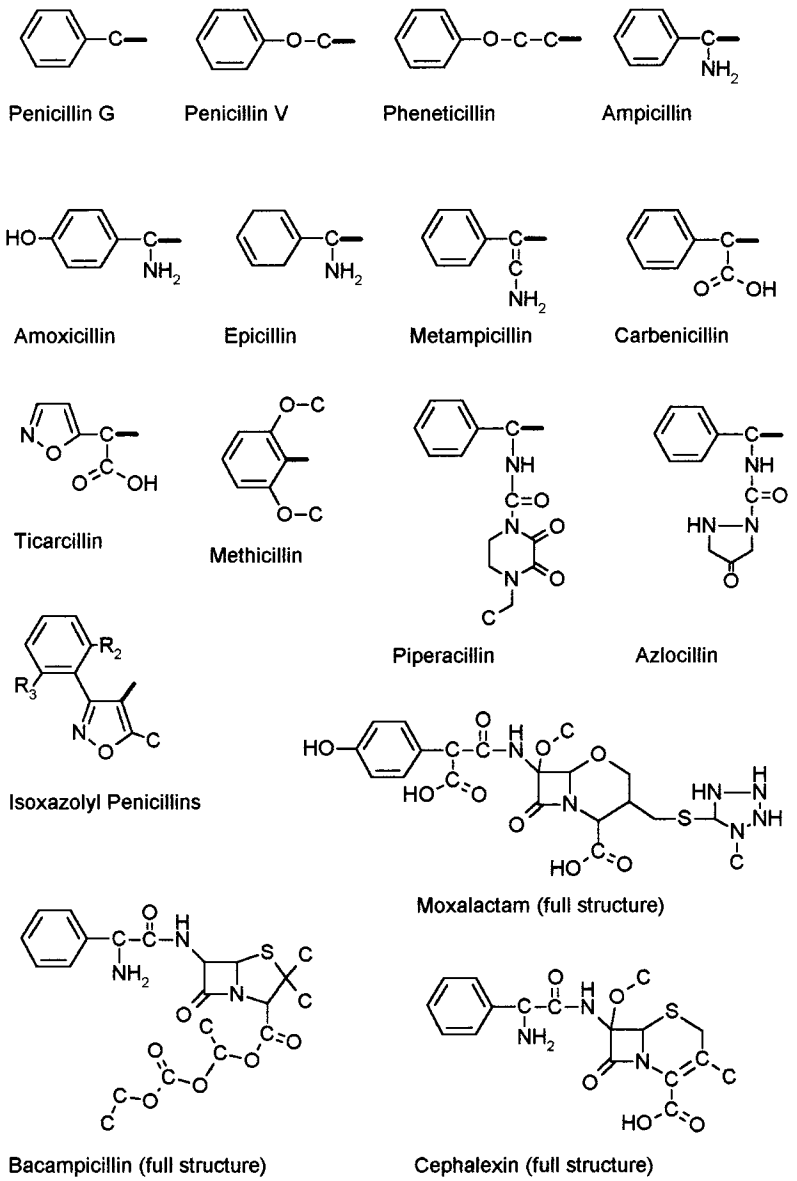

Fig. 2 Side chain residues (Fig. 1) of the penicillins tested (isoxazolyl penicillins: oxacillin, R2 = R3 = H; cloxacillin, R2 = Cl, R3 = H; dicloxacillin, R2 = R3 = Cl). For bacampicillin, moxalactam, and cephalexin, full structures are shown.

l^{-1} sodium caseinate. Enzyme substrate dilution consisted of 0.2 mol l^{-1} potassium citrate buffer (pH 3.9) containing 0.003 mol l^{-1} H_2O_2 and 0.001 mol l^{-1} TMB (100 μl per well). The enzyme reaction stop solution was 1 mol l^{-1} H_2SO_4 (100 μl per well). Colour development of the EIA reaction was measured with an AT 400 microtitre plate reader (SLT, Crailsheim, Germany). The absorbance data were evaluated using a competitive EIA calculation software.[16]

Synthesis of conjugates

The immunogen (ampicillin–KLH conjugate) was prepared by glutaraldehyde reaction. Ampicillin (10 mg) and KLH (1.67 mg) were dissolved with 2 ml PBS. Glutaraldehyde solution 25% (40 μl) was added, and the mixture was stirred at ambient temperature for 4 h. Then, the ampicillin–KLH conjugate was dialyzed against 3 × 5 l PBS for 3 d and stored at −80 °C until use.

For the synthesis of the labelled antigen, ampicillin and metampicillin, respectively, were conjugated to HRP with EDPC as previously described.[15]

Immunization

Twelve-week-old female mice [six each BALB/c strain and a hybrid strain of BALB/c × (NZW × NZB)] were immunized by intraperitoneal injection of 33 μg of ampicillin–KLH conjugate, dissolved in PBS and emulsified in Freunds complete adjuvant (1 + 2). The first booster injection, using the same composition and amount of antigen, was given 11 weeks after the primary injection. At day 138 the mice received a second, subcutaneous booster injection of 40 μg ampicillin–KLH conjugate emulsified in Freunds incomplete adjuvant. Finally, at day 189, three days before fusion, the animals got a final booster injection of 70 μg of antigen in PBS alone.

Table 1 Characteristics of the monoclonal antibodies (Mab) against ampicillin and of the rabbit antiserum presented previously[15]

	Mab 1D1	Mab 3B5	Rabbit antiserum
Immunglobulin subclass	IgG_{2a}	IgG_1	—
50% Inhibition concentration/ng ml^{-1}	50	26	42
Detection limit[a]/ng ml^{-1}	17	12	14
% Cross-reactivity with			
Ampicillin	100	100	100
Amoxicillin	187	108	100
6-APA	1.0	0.4	27
Azlocillin	14	5.0	244
Bacampicillin	1.9	1.7	1.5
Carbenicillin	1.5	<0.3	86
Epicillin	160	130	51
Cloxacillin	30	4.8	108
Dicloxacillin	44	1.7	127
Metampicillin	25	19	288
Methicillin	10	0.6	71
Moxalactam	<0.7	<0.3	2.9
Oxacillin	14	4.5	218
Penicillin G	31	8.6	222
Penicillin V	33	10	355
Pheneticillin	52	2.4	150
Piperacillin	35	11	58
Ticarcillin	3.5	0.5	244
Ampicillin hydrolyzed	<0.7	<0.3	<0.3
Penicillin G hydrolyzed	<0.7	<0.3	<0.3

[a] Calculated from the ampicillin concentration giving 30% inhibition binding

Direct enzyme immunoassay

For the determination of the relative antibody titre of the mice sera, and for detection of antibody secreting hybridomas a non-competitive EIA system was used. Microtitre plates were coated overnight at room temperature with anti-mouse immunoglobulins (10 μg ml^{-1}). Then, free binding sites were blocked for 30 min. Subsequently, serially diluted mouse serum or hybridoma culture supernatant (100 μl per well) were added and incubated for 1 h. After a washing step ampicillin-HRP was added (2.2 μg ml^{-1}; 100 μl per well) and incubated for 1 h at room temperature. The plate was washed again, and enzyme substrate solution was added (100 μl per well). After 20 min the enzyme reaction was stopped, and colour development was measured.

To check the sensitivity and specificity of the monoclonal antibodies produced, a competitive direct EIA was used. In a similar test system based on a double antibody solid phase (as described above), first appropriate dilutions of cell culture supernatants (1 + 499 for Mab 1D2 and 1 + 399 for Mab 3B2) were added, and after a washing step ampicillin–HRP (0.22 and 0.275 μg ml^{-1}, respectively; 50 μl per well) was incubated simultaneously with standard solutions of β-lactam antibiotics. The relative cross-reactivity (ampicillin = 100%) of each antibiotic was calculated on the basis of the concentration necessary to inhibit 50% binding of the labelled antigen.

Hybridoma production

Hybridoma and myeloma cells (X63-Ag8.653) were maintained in Dulbecco's modified Eagle medium supplemented as previously described.[17] For fusion, a single-cell suspension from spleen and axillary lymph nodes of a hyperimmunized mouse was made and fused with myeloma cells according to Fazekas de St. Groth and Scheidegger[18] in a ratio of 2 : 1. Culture supernatants were tested for ampicillin-specific antibodies 12 d after fusion in the non-competitive direct EIA as described above. Positive hybridomas were cloned at least three times by limiting dilution techniques. Selected antibodies were mass-produced in a Mini-Perm bioreactor (Heraeus Instruments, Osterode, Germany) and subsequently purified by affinity chromatography on protein A–agarose (BioRad, München, Germany).

Preparation of immunoaffinity columns

Purified Mab 1D1 against ampicillin and a previously described[10] antibody against cloxacillin (Mab 1F7) were each coupled to CNBr-activated Sepharose 4B according to the instructions of the manufacturer (Pharmacia, Freiburg, Germany). The amount of antibody used in the coupling procedure corresponds to the amount of antibody bound per ml of sepharose (Table 2). For preparation of IA columns, portions of the gels (250 μl of the 1D1-IA gel and 100 μl of the 1F7-IA gel) were filled into disposable minicolumns (MobiTec, Göttingen, Germany).

Table 2 Antigen binding capacities of the produced immunosorbents used for recovery experiments

Monoclonal antibody	Antibody bound/ ml^{-1} of gel	Antigen bound/ ml^{-1} of gel	Remaining activity of bound antibody (%)
AMPI 1D1	5.39 mg	6.6 μg	26.3
CLOXA 1F7	1.80 mg	5.4 μg	49.2

Determination of antigen binding capacity of the IA columns

For capacity determination of the IA columns, 40 ml of PBS containing ampicillin and cloxacillin, respectively, each at a concentration of 100 ng ml^{-1}, were applied to the respective column at a flow rate of approximately 2–3 ml min^{-1} using disposable syringes. Then, the columns were washed with 10 ml PBS, and bound β-lactam antibiotic was eluted with either 3 ml of glycine–HCl buffer (0.1 mol l^{-1}; pH 2.5) or 2 ml of methanol. All fractions (*i.e.,* antibiotic solution passed through the IA column, wash solution, and eluate) were collected, and the penicillin content was quantitatively determined by using EIAs based on either polyclonal antibodies against ampicillin[15] or Mabs against cloxacillin.[10] For this purpose, the eluates were diluted with PBS.

Performance testing of the IA columns

For the determination of recovery rates of the IA method, PBS containing penicillins at varying concentrations were applied to the columns (flow rate: 2–3 ml min^{-1}). IA columns based on Mab 1F7 were tested with cloxacillin and oxacillin, whereas recovery studies for ampicillin, amoxicillin, cloxacillin, dicloxacillin, penicillin G and oxacillin were performed with the IA columns based on Mab 1D1.

Results and discussion

The aim of this study was to produce monoclonal antibodies with group specificity for the penicillin group of the β-lactam antibiotics. Based on promising experiences with ampicillin–protein conjugates for the production of group-specific polyclonal antibodies,[15] we focussed on ampicillin conjugates as immunogens. Unfortunately, the previously described ampicillin–BSA conjugate, as well as an ampicillin–glucose oxidase conjugate coupled by reductive alkylation (details not shown), failed to induce a specific antibody response in mice. However, in the sera from three out of 12 mice immunized with the ampicillin–KLH conjugate, antibodies reactive with the ampicillin–HRP were detectable in a non-competitive EIA. In a competitive EIA, the conjugate binding of the sera from two of the mice (both of the hybrid strain) could be inhibited by addition of free ampicillin. These findings are in accordance with other published results,[8,15] indicating that penicillin conjugates with an intact β-lactam ring seem to have a generally low immunogenicity, whereas immunizations with penicilloyl protein conjugates result in high antibody titres.[19] Anti-penicilloyl antibodies, however, are of little value for residue analysis, since they mainly react with the open ring form, while MRLs are set for the parent compounds.[1]

Both mice producing specific antibodies against ampicillin were selected as the spleen donors for cell-fusions. When cell culture supernatants were first screened by EIA, 28 out of 736 wells recorded an absorbance value of >2.0. Eleven clones, all secreting specific antibodies from the IgG type, could be established. Based on the results of preliminary competition studies, two of them, designated AMPI I 1D1 and AMPI II 3B5, were chosen for further characterization. The other antibodies which were not further characterized exhibited 50% inhibition values for ampicillin in the range from 60 to 400 ng ml^{-1}.

Table 1 shows the characteristics of the two selected Mabs 1D1 and 3B5. Both antibodies were most specific for amphoteric penicillins (ampicillin, amoxicillin, and epicillin), indicating that the intact penicillin nucleus and the primary amino group at the side chain of these penicillins are the most important structures for antibody binding. This hypothesis is supported by the findings that presence of additional side chains at the 6-APA nucleus (*e.g.*, moxalactam, bacampicillin), cleavage of the β-lactam ring (*e.g.*, hydrolyzed ampicillin), and replacement of the amino group by a carboxyl group (*e.g.*, carbenicillin) all led to a significant decrease in cross-reactivity. Furthermore, the two tested compounds of the cephalosporin class (having a six-membered thiazolidine ring condensed with the β-lactam ring) showed no detectable cross-reactivity.

However, the two Mabs behaved completely differently concerning the reactivity with the isoxazolyl penicillin antibiotics (cloxacillin, dicloxacillin, oxacillin) and penicillin G, which are the most commonly used penicillins next to ampicillin and amoxicillin in veterinary medicine. Mab 1D1 reacted strongly with all of these substances (30 to 44%) except for oxacillin, whereas Mab 3B5 exhibited only minor cross-reactivity (1.7 to 8.6%). Considering the poor affinity of both Mabs for free 6-APA, it is obvious that substitution of the 6-amino group plays an important role concerning cross-reactivity of the antibodies. In detail, major structural differences of the side chain, in comparison with ampicillin, drastically reduce reactivity of the Mab 3B5. In contrast, specificity tests using a polyclonal rabbit antiserum,[15] which exhibited extraordinarily high cross-reactivities to most of the tested penicillins, showed that the intact and unmodified penicillin nucleus is the most important epitope for antibody binding. Mab 1D1, however, has an intermediate position between Mab 3B5 and the polyclonal antibodies in aspects of specificity. This is indicated by the relatively high cross-reactivities with amoxicillin and epicillin (compared with the polyclonal antiserum) and with the other penicillins under study (compared with Mab 3B5).

With regard to EIA sensitivity both monoclonal antibodies showed 50% inhibition values for ampicillin similar or even better than the previously described rabbit antiserum.[15] Furthermore, both Mabs also had higher affinities for amoxicillin (50% inhibition dose 25 ng ml^{-1} and 28 ng ml^{-1}, respectively). The intra-assay RSD for standard concentrations were usually below 7%. Standard curves for some of the penicillins which are regulated by MRLs within the European Union are shown in Fig. 3. The detection limits of the standard curves were in the range from 12 ng ml^{-1} to 130 ng ml^{-1}. In order to determine the effect of heterologous labelled antigen on test sensitivity, a HRP conjugate of metampicillin was prepared. However, the 50% binding values indicated that no sensitivity improvement could be achieved with this conjugate (data not shown). Thus the sensitivity of the assay is not sufficient for the detection of the penicillins in milk at the proposed MRL levels, but the Mabs could be useful for the detection of ampicillin, amoxicillin and the isoxazolyl penicillins in meat and meat products due to the

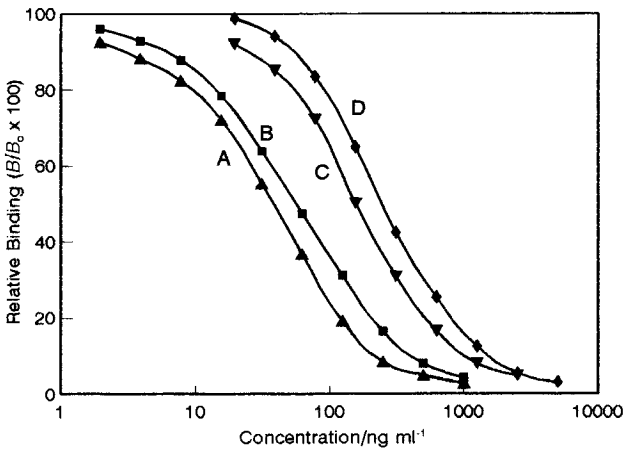

Fig. 3 Standard curves for some penicillin antibiotics (A, amoxicillin; B, ampicillin; C, penicillin G; and D, cloxacillin) in the competitive enzyme immunoassay using monoclonal antibody 1D1. The absorbance values of the negative control solution (B) were 0.9–1.1 units. The RSD were usually below 7%.

Table 3 Recovery of penicillin antibiotics from the 1D1-immunoaffinity columns. Eluates were analyzed by enzyme immunoassays[a]

Antibiotic added	Sample volume and concentration	Antibiotic recovered				
		Mean/ng	*s*/ng	s_r (%)	Recovery (%)	*n*
Ampicillin		501.3	17.4	3.5	100.3	4
Amoxicillin	5 ml PBS	473.4	43.5	9.2	94.7	5
Cloxacillin	containing 100 ng ml^{-1}	347.0	na	na	69.4	1
Dicloxacillin	antibiotic	361.5	na	na	72.3	1
Penicillin G		341.3	35.7	10.5	68.3	3
Oxacillin		336.5	na	na	67.3	1
	10 ml PBS					
Dicloxacillin	containing 10 ng ml^{-1} dicloxacillin	90.3	8.0	8.9	90.3	4

[a] *s*, Standard deviation; s_r, relative standard deviation; and *n*, number of replicates; na = not applicable.

Table 4 Specificity of the monoclonal antibody 1F7 and recovery of isoxazolyl penicillins from the 1F1-immunoaffinity columns: 20 ml buffer solution containing 10 ng isoxazolyl penicillin per ml were applied to the column[a]

Penicillin	Antibody characteristics		Isoxazolyl penicillin recovered (n = 5)			
	Relative cross-reactivity (%)[b]	Estimated K_d[c]/nmol L^{-1}	Mean/ng	*s*/ng	s_r (%)	Recovery (%)
Cloxacillin	100	5	201.4	18.8	9.3	100.7
Dicloxacillin	329.9	2	nd	nd	nd	nd
Oxacillin	11.0	47	197.7	18.0	9.1	98.8

[a] *s*, Standard deviation; s_r, relative standard deviation; *n*, number of replicates, Nd = Not determined. [b] Determined under the conditions of an indirect competitive EIA.[10] [c] Dissociation constant calculated from the concentration required for 50% inhibition binding in an indirect, competitive EIA.[10]

higher MRLs set for these foodstuffs (50 μg kg^{-1} and 300 μg kg^{-1}, respectively).

Next to the development of EIAs for screening purposes, the use of monoclonal antibodies for the production of immunoaffinity columns finds increasing interest in the area of residue analysis in food safety. Quality and usefulness of IA columns primarily rely on the affinity, characterized by the dissociation constant K_d, and the specificity of the employed antibodies. Besides these parameters, antigen binding capacity is another important factor and therefore use of monoclonal antibodies has advantages over polyclonal antisera. Since MRLs have been set for eight penicillins within the European Union, only the preparation of multi-immunoaffinity chromatography (MIAC) columns, based either on different antibodies or on one antibody with broad specificity, seems to be a useful approach. Therefore the Mab 1D1 and a previously described monoclonal antibody, designated 1F7,[10] which reacted with all isoxazolyl penicillins, were used for the preparation of immunoaffinity columns. The results of the capacity studies, as measured by EIA, are presented in Table 2. Between 5.4 and 6.6 μg of the respective β-lactam antibiotic were bound per ml of gel, which corresponds to a calculated specific activity of the bound antibodies of 26.3 and 49.2%. Taking into account that the antibodies were attached to the gel support by random coupling *via* primary amines, which may result in inactivation of the antibodies due to coupling at or near the antigen binding site (steric hindrance), the remaining specific activity of 49.2% represents a good result, but even 26.3% are acceptable.[6,20] The produced immunosorbens were found to be stable at 4 °C for at least six months, whereas the specific activity of egg yolk antibodies coupled to CNBr activated sepharose disappeared within 4 days.[8]

Bound ampicillin could be completely eluted from the 1D1-IA columns (250 μl gel) using 3 ml of glycine–HCl buffer. This also enabled the repeated use of the columns, as tested with ampicillin buffer solutions. Moreover, the mild elution conditions also make it possible to use these columns for on-line analyte clean-up and enrichment within an HPLC detection system for penicillins. In contrast, complete desorption of bound cloxacillin from the 1F7-IA columns (100 μl gel) only could be achieved by methanol, due to the higher affinity of the employed Mabs.

In a preliminary study, recovery of the most commonly used penicillins from the 1D1-IA columns was tested at a concentration of 100 ng ml^{-1} (total 500 ng; antigen binding capacity 1,650 ng per column). The results (Table 3) showed that the recovery rates varied with the antibody affinity for the respective antibiotic (see Table 1). For instance, 100% recovery was achieved for ampicillin, whereas only 68.3% of the penicillin G was found in the eluate after the IA procedure. However, besides antibody affinity, the performance of immunoaffinity chromatography is also influenced by the concentration of antibody binding sites (column capacity) and the applied antigen concentration.[6] This means that at higher column capacities (*e.g.*, increasing gel volume) or lower antigen concentrations recovery increases significantly. Consequently, recovery of dicloxacillin increased from 72.3% to about 90% (Table 3) when this antibiotic was applied to the IA column at a concentration of 10 ng ml^{-1}. Based on these findings it could be assumed that the capacity of the produced IA column and affinity of the employed antibody should be sufficient for the isolation of penicillins from milk and animal tissues at the MRL levels.

As expected from the specificity studies and the resulting estimate of the dissociation constants, cloxacillin and oxacillin were retained by the 1F1-IA columns to a high degree (Table 4). Detailed recovery studies for dicloxacillin were not performed, but due to the extraordinarly high affinity of the Mab 1F7 for this isoxazolyl penicillin it can be assumed that this antibiotic also is retained by the IA columns. Thus, besides the group-specific 1D1-IA column a further column specific for the isoxazolyl penicillins was produced. Combining both immunoaffinity gels in one column could be advantageous in terms of recovery and reproducibility in future application studies.

In conclusion, this is the first report about production of high-affinity monoclonal antibodies reactive with all penicillins frequently used in veterinary medicine. The usefulness of these antibodies for the preparation of immunoaffinity columns for this group of antibiotics could be demonstrated. Detailed studies

on the applicability of these columns for clean-up of penicillins from food combined with an HPLC analysis are currently in progress.

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