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Development of an Antibody Hapten-Chip System for Detecting the Residues of Multiple Antibiotic Drugs*

ABSTRACT: The abuse of antibiotic drugs during animal production remains a worldwide problem and the subsequent detection of the residues of various drugs present at low concentrations in complex biological matrices poses significant analytical challenges. The present study outlines a practical biochip assay system to identify antibiotic residues in different animal tissue extracts. The system uses a simple but efficient multiresidue sample extraction procedure to isolate the antibiotic residues which were then identified directly using high-affinity monoclonal antibodies presented in a competitive immunoassay with conjugated antibiotic hapten-chips. The hapten-chip can analyze six samples each for eight antibiotics on a single chip within 3 h. The analytical results with both artificial positive standard samples and the incurred samples show that the antibody hapten-chip system has a comparable accuracy and a similar sensitivity to a standard ultra performance liquid chromatography–mass spectrometry (MS)/MS assay. In conclusion, an effective analytical screening system based on antibody hapten-chip was developed for detecting multiple antibiotic residues from multiple samples.

KEYWORDS: forensic sciences, antibiotics, residue, chip, immunoassay, sample extraction

Antibiotics are used extensively throughout the world in the production of animal products for human food consumption. Numerous potential effects on human health are associated with the use of such antibiotic residues, including the risk of increased resistance of pathogenic bacteria towards the antibiotics and adverse human health effects after consumption (1) as well as inhibition of starter cultures used in cheese and yogurt production (2).

To protect the health of consumers, some supranational governmental organizations such as the European Union (EU), the Food and Agriculture Organization of the United Nations (FAO), and the World Health Organization (WHO) as well as many countries have set the legal maximum residue levels (MRL) for some antibiotics in foodstuffs containing animal products (3). For example the EU has imposed a mandatory MRL of 500 μg/kg for streptomycin (STR) and 100 μg/kg for certain sulfonamides (SAs) in pig muscle samples. To enable adequate risk evaluation and guide subsequent action, sophisticated and robust analytical methods are needed to screen for a wide variety of antibiotic residues that may be present due to the choice of animal feed. The highest analytical level and conventional technique for the analysis of illegal veterinary drug residues for limited numbers of samples is liquid chromatography combined with tandem mass spectrometry (liquid chromatography–mass spectrometry [LC–MS]/MS) (3). This provides full (or complementary) information to enable the unequivocal molecular identification of target compound(s). However, other rapid methods are also needed for screening when large numbers of samples have to be monitored for different antibiotics as both high throughput of samples and high speed of analysis are of the essence.

Previously, immunological separation assays have been successfully employed for the detection of veterinary drug residues in animal products with good specificity and sensitivity. In 1991, Ekins (4) suggested that quantitative immunoassays could be developed using microspots of antibodies on nonporous solid supports, which would permit the development of ''multi-analyte'' immunoassay systems which might provide sensitive measurements of multiple analytes simultaneously. Recent advancements in biochip technology have demonstrated the practicality of this approach, using protein chips for the sero-diagnosis of various diseases (5–8). We have also reported on the development of monoclonal antibody-based protein chip systems for detection of small molecules and demonstrated its use for detection of drugs in urine samples, illustrating its application for drug abuse enforcement (9–11).

Here we report on the development of an antibody hapten-chip system for high-throughput testing and routine screening of antibiotic residues in animal tissues, and potentially for screening food containing such contaminated tissues. The detection chip is based

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on some five pairs of ovalbumin (OVA)-conjugated antibiotic antigens, which are immobilized at discrete locations on the surface of agarose-modified glass slides. The assay is a competitive immunoassay (10,11) where the fluorescent signal for each particular drug is decreased when the drug is present in the test sample as it competes with drug immobilized on the hapten-chip (OVA–drug) for interaction with the specific antidrug antibody. The pattern of fluorescence signals are imaged using a confocal microarray scanner and an analysis algorithm provides a quantitative estimation of the drug. The specificity of the antibodies employed for interaction with their antigen is one of the important aspects of these systems, and we have examined the cross-reactivity of the assay with several other common antibiotic analytes. Cross-reactivities between individual antibodies and other drugs belonging to the same class as their target analyte were discovered at different levels. As expected, the antibody hapten-chip system was found to be suitable for initial high-throughput screening for the presence of some eight different veterinary antibiotics with high sensitivity and high specificity for classes of analytes. To aid this high-throughput screening system we also developed a simple multiresidue sample extraction procedure allowing the simultaneous purification of several different drugs from different animal tissues with good recoveries and high repeatability. The combination of a simple multiresidue extraction procedure and a hapten-chip assay system is commended for preliminary screening of multiple samples, allowing efficient redirection of positive samples for full LC–MS/MS analysis.

Materials and Methods

Standards, Conjugates, Antibodies, and Reagents

Eight antibiotics standard reference materials: enrofloxacin (ENR), STR, sulfamethazine (SM2), sulfaquinoxaline (SQX), sulfamethoxazole (SMZ), sulfisoxazole (SIZ), sulfamonomethoxine (SMM), and dihydrostreptomycin (DSTR) were obtained from Laboratorium Dr. Ehrenstorfer (Augsburg, Germany). Five drug conjugates (drug–bovine serum albumin [BSA] or drug–OVA) and their monoclonal antibodies were prepared in our laboratory or purchased from the Wang'er Corporation (Beijing, China). These OVA conjugates were used as the substrates for printing to manufacture the hapten-chips. Cy3-labeled goat antimouse IgG and goat antirabbit IgG were purchased from Amersham Biosciences (Piscataway, NJ). The extraction buffer that is included in the Veterinary Drug Residue Detection Array Kit (Cat. No. 310010) and the Cy3-labeled BSA were obtained from CapitalBio Corp. (Beijing, China). All other chemicals used were analytical grade and were purchased from the Beijing Chemical Reagents Company (Beijing, China).

Instrumentation

The EcoSampler™ Analyte Extraction Station, the printing robot SmartArrayer™-48 and the LuxScan™-10K/B laser confocal scanner were provided by CapitalBio. The ultra performance (UP) LC–MS/MS was carried out on a tandem quadrupole mass spectrometer (Quattro Premier XE; Waters Micromass, Manchester, U.K.) interfaced with an Acquity UPLC System (Waters, Milford, MA).

The Specificity of Antibodies

The specificities of the five antibodies were assessed by determining the extent to which each cross-reacted with analyte compounds of a similar chemical structure to that used to raise the antibody. The potentially cross-reacting analytes were added into an analyte-free matrix to give a wide range of suitable concentrations. The cross-reactivity is defined as the comparison of the concentration of analyte (A) that causes a 50% reduction in signal with the concentration of a cross-reactant (CR) that has the same effect (A/CR × 100%).

Preparation of Artificial Positive Standard Tissue Samples and Multiresidue Sample Extraction Procedure

Antibiotic-free tissue samples (pig muscle, pig liver, chicken muscle, and chicken liver) were obtained at slaughter from animals reared in an experimental unit without antibiotic treatments. The tissues were blended using the EcoSampler™ Analyte Extraction Station. An accurately weighed 5.0 ± 0.05 g of the minced tissue was placed in a 50 mL centrifuge tube and added with 50 μL of appropriate concentrations of the antibiotic solutions and mixed thoroughly also in the EcoSampler™ Analyte Extraction Station.

The drugs were extracted by introducing 10 mL of the extraction buffer, vortexing for 2 min, and then incubating for 20 min in an 80°C water bath using the EcoSampler™ Analyte Extraction Station. The tube was then centrifuged at 2500 × *g* for 10 min. To avoid possible fat contamination, the clear supernatant in the middle layer was carefully decanted into a clean centrifuge tube, adjusted to pH 7.0 with 2 M NaOH, and then centrifuged at 2500 × *g* for 10 min. The supernatant was then diluted three times with 0.01 M phosphate-buffered saline (PBS) making a total of *c.* nine times dilution of the original tissue concentration. This supernatant was ready for analysis by the hapten-chip.

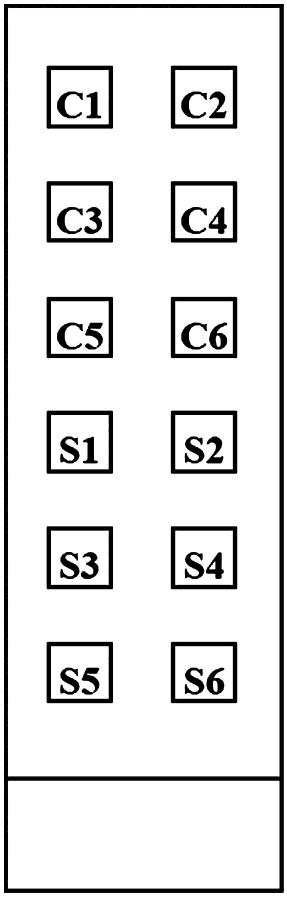
Printing of Hapten-Chips

A printing robot SmartArrayer™-48 with a SMP3 stealth microspotting pin (TeleChem International, Sunnyvale, CA) was used to print the drug–OVA conjugates onto activated agarose surface-modified glass slides with spot sizes about 180 μm in diameter. The slides were dried in the vacuum chamber at 37°C for 16 h and could be stored at room temperature for up to 6 months. Twelve 5 × 9 arrays of OVA-conjugated drugs as well as various controls were printed in a 2 × 6 grid pattern on each slide (Figs. 1*A* and 1*B*). Each drug–OVA and control spot were arrayed in triplicate on the slide (boxed areas in Fig. 1*C*). The controls include mouse IgG as second antibody control (external control [EC]), rabbit IgG as fluorescence density value normalization index (data process/normalization control [DC]), 1 mg/mL OVA as negative control (NC), printing buffer as blank control, 1 mg/mL Cy3-BSA as protein immobilization control (quality control [QC]), and a nonrelevant drug–OVA conjugate as a sample extraction control (SC).

All of the EC spots were expected to be positive from reaction of the immobilized mouse IgG control with the Cy3-labeled goat antimouse conjugate used in the assay. Similarly for DC, which reacts with the Cy3-labeled goat antirabbit conjugate. These six arrayed controls were used to prepare a standard curve while the lower six arrayed conjugates were used for the assay of samples. Each slide could be compartmented using a gasket system (CapitalBio) into six individual reactions for the presence of eight different antibiotics, allowing up to six different tissue samples to be analyzed in parallel.

Immunoassay Procedure

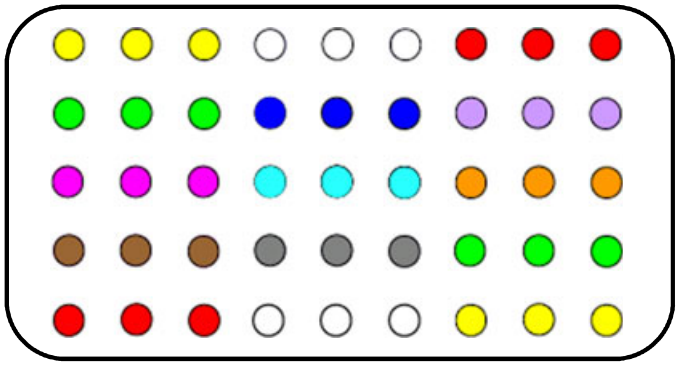
The immunoassay procedure was as follows: the prepared chips were blocked with 100% sheep serum at room temperature for 30 min and then rinsed three times with PBS, 0.05% Tween 20,



A

QC	BC	EC
DC	SM2	SMZ
SC	SQX	ENR
STR	NC	DC
EC	BC	QC

B



C

FIG. 1—*The typical layout of the chip. (A) Schematic showing the glass slide and the polyester frame forming the reaction chambers above each array. (B) Layout of the array. (C) Image of one of the 5 × 9 arrays (each drug/substance is printed in triplicate).*

pH 7.4 (PBST). A mixture of 10 μL primary antibodies and 10 μL standard curve reference solution or the sample extracts were added onto the sample grids, which was then maintained at 37°C in a humid box for 30 min. The chip was then rinsed three times with PBST, the secondary antibodies (Cy3 goat antimouse IgG and Cy3 goat antirabbit IgG) were added and the chip was incubated at 37°C for 30 min. The chip was then rinsed three times with PBST and after centrifugal drying the surface was then ready for image scanning. The entire test from tissue homogenate to data took *c.* 3 h for six samples to be analyzed on a single chip. Typically, the expected three test spots for each drug bound the antidrug antibody, and the Cy3-labeled second antibodies reacted with captured antidrug antibodies to provide a fluorescent signal. The DC, EC, and SC spots also provide fluorescent signals.

Chip Scan and Data Evaluation

The fluorescent signals were detected by a LuxScan™-10K/B Laser Confocal Scanner and analyzed using VetCode software (CapitalBio) to generate a quantitative representation of the different detectable antibiotics. In order to evaluate the data, the fluorescence median of six DC control spot signals of rabbit IgG within the same array were calculated, and these data were used as a normalization index to adjust for experimental variation between different arrays on the same chip and between different chips. The mean values of the fluorescence obtained for the standards and the samples were divided by the mean fluorescence value of the zero standards and multiplied by 100. The zero standards were thus made equal to 100% and the fluorescence values of subsequent samples were then given as a percentage of the zero standard. For each chip, a standard curve was generated in parallel with the sample analysis and subsequently used for the quantification of each drug residue in the sample. The values calculated for the standards were entered in a system of coordinates on a semilogarithmic graph against the drug concentration in μg/kg. The calibration curve should be linear within the given range, and the drug equivalents in μg/kg corresponding to the fluorescence of each sample can then be read from the calibration curve.

Results

Hapten-Chip and UPLC–MS/MS Comparisons

Comparison of the performance of the antibody hapten-chip system with the standard UPLC–MS/MS assay (with reference to Chinese National Standards: GB/T 20756–2006, GB/T 20751–2006, GB/T 20759–2006 and Industrial Standard: SN/T 1925–2007) provides an estimate of the sensitivity of this new system. Five chicken muscle samples containing a range of concentrations of added SMZ residues were analyzed by both the hapten-chip and

TABLE 1—*The concentrations of sulfamethoxazole (SMZ) determined by the antibody hapten-chip system compared with the UPLC–MS/MS analysis for chicken muscle.*

Added concentration, μg/kg	Hapten-chip* (μg/kg)	UPLC–MS/MS, μg/kg
10	8.7 ± 2.3	16.8
20	17.4 ± 3.3	21.9
40	34.5 ± 4.0	32.1
60	47.8 ± 6.9	63.5
80	68.6 ± 11.7	70.4

UPLC, ultra performance liquid chromatography; MS, mass spectrometry.

*Mean ± SD; $n = 9$

UPLC–MS/MS (Table 1). The correlation coefficient (r) for the hapten-chip and UPLC–MS/MS was 0.96 ($t = 5.945$, $p < 0.005$), indicating the comparability of these two methods for quantifying SMZ.

Cross-Reactivities with Other Analytes

There are a number of other antibiotics commonly used in commercial animal husbandry which might be potentially encountered in tissue samples. The cross-reactivities of some other analytes of the same class as the target analytes are shown in Table 2 for each of the five antibodies used. DSTR can be detected by the STR antibody with 130% cross-reactivity. In contrast, the SMZ antibody showed 90% cross-reactivity with SIZ and 50% with SMM. The high cross-reactivity means that the presence of SIZ and SMM in tissues would be indicated by the SMZ antibody. Thus, although the hapten-chip was designed to specifically detect five antibiotics, the presence in tissue samples of eight different antibiotics could be indicated.

Standard Curves and the Limit of Detection for the Assay System

When a drug was present in the sample the fluorescent signal at the corresponding array location was decreased, as the drug in the sample competed with drug–conjugate immobilized on the hapten-chip for the antidrug antibody. The decrease in fluorescent signal was found to be proportional to the amount of drug in the sample, within a determined linear measurement range. If the concentration of the drug in an unknown sample was the maximum of the working range (or close to it) after initial analyses, the sample would be diluted 10-fold with extraction buffer for secondary analyses until the concentration was in the linear measurement range.

A series of standard solutions of each of the test drugs was prepared in extraction buffer and the linearity and IC_{50s} (the 50% inhibitory concentration) were determined. The calibration curves measured using the hapten-chip are shown in Fig. 2. As can be seen, excellent linearity was acquired for all five drugs indicating the good quality of the calibration curves. The IC_{50} values for all five drugs were calculated. The limit of detection (LOD) (mean + 3SD) of the assay was determined by the analysis of four tissue species (chicken muscle, chicken liver, pig muscle, and pig liver), each with 20 samples known to be free of antibiotic residues. The IC_{50} and LOD for each of the five drugs are summarized in Table 3. The IC_{50} and LOD for SIZ, SMM, and DSTR could be estimated by the antibody cross-reactivity as they have the same extraction efficiencies.

Precision and Accuracy of the Analysis of Artificial Positive Standard Tissue Samples

The method was validated in several tissue samples including chicken muscle, chicken liver, pig muscle, and pig liver with each drug added at different concentration levels. An example of hapten-chip signals seen with different amounts of standards added to chicken muscle samples is shown in Figs. 3*A–C* and the recovery and repeatability of the method was determined (Table 4). The mean recoveries were in the range of 70–110% for all of the analytes in each of the tissue samples. The coefficients of variation were each ≤25%, which showed good reproducibility for all eight antibiotics under MRL levels.

Analysis of Incurred Material

Tissue samples from animals exposed to antibiotics were used to examine the performance of the antibody hapten-chip system. Four

TABLE 2—*The cross-reactivity of related analytes with the five antibodies.*

	Antibody				
Analyte	Sulfamethazine	Sulfaquinoxaline	Sulfamethoxazole	Enrofloxacin	Streptomycin
Sulfamethazine	100%	–	–	–	–
Sulfamonomethoxine	–	–	50%	–	–
Sulfadimethoxine	20%	17%	–	–	–
Sulfaquinoxaline	–	100%	–	–	–
Sulfadiazine	–	–	21%	–	–
Sulfamethoxazole	–	–	100%	–	–
Sulfaguanidine	–	–	–	–	–
Sulfisoxazole	–	–	90%	–	–
Sulfamethoxypyridazine	–	–	–	–	–
Sulfamerazine	8%	–	4%	–	–
Enrofloxacin	–	–	–	100%	–
Ciprofloxacin	–	–	–	1%	–
Norfloxacin	–	–	–	–	–
Chlorampheicol	–	–	–	–	–
Thiamphnicol	–	–	–	–	–
Dihydrostreptomycin	–	–	–	–	130%
Streptomycin	–	–	–	–	100%
Neomycin	–	–	–	–	–

Entries without values denote a cross-reactivity rate of less than 1%.

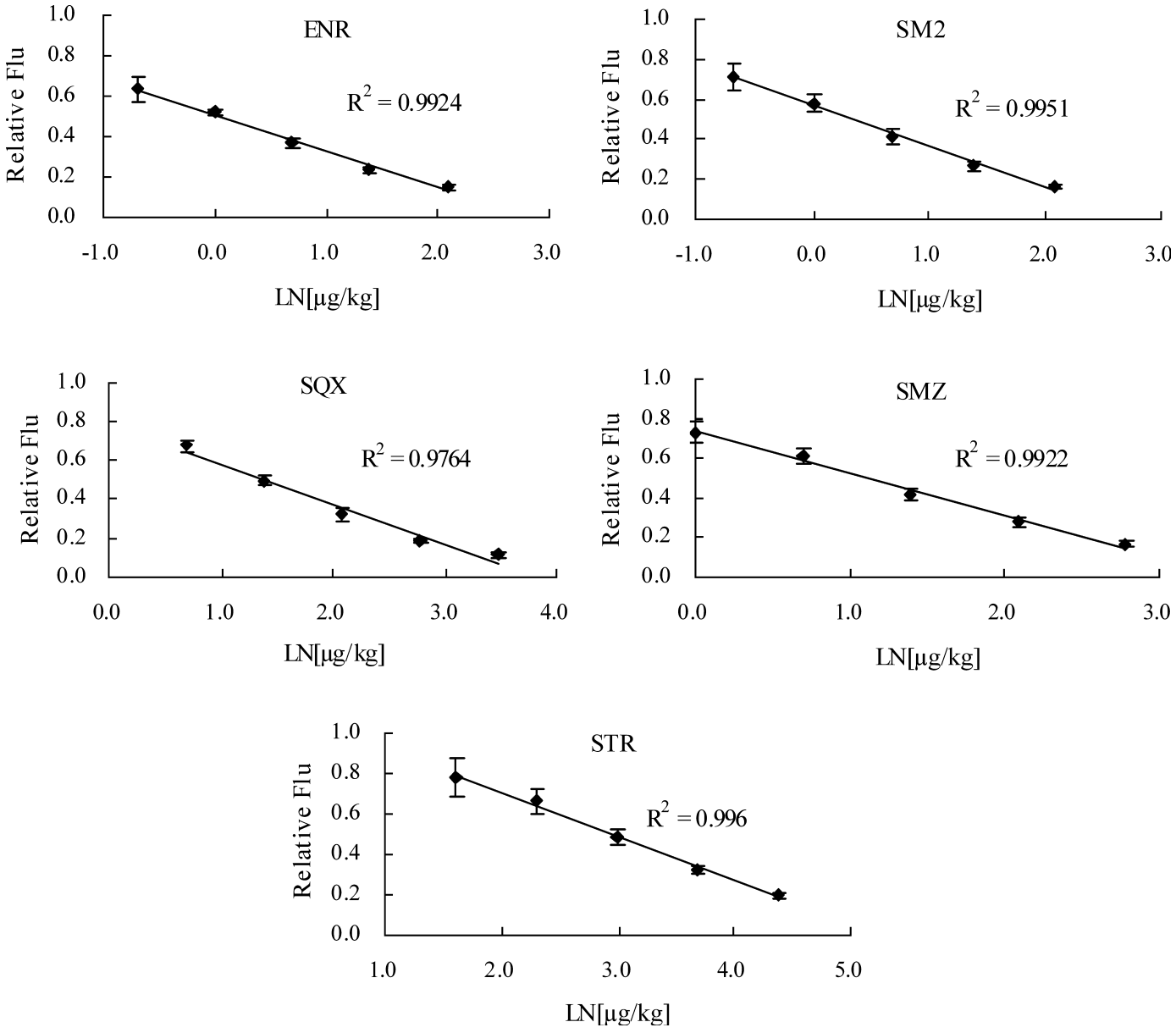


FIG. 2—*Typical calibration curves for five drugs obtained using the antibody hapten-chip system. ENR, enrofloxacin; SM2, sulfamethazine; SQX, sulfaquinoxaline; SMZ, sulfamethoxazole; STR, streptomycin.*

TABLE 3—*The IC_{50} and limit of detection of the five drugs by the antibody hapten-chip system.*

Name of drug	IC_{50} (μg/kg)*	LOD† (μg/kg) Chicken muscle	Chicken liver	Pig muscle	Pig cliver
sulfamethazine (SM2)	1.4 ± 0.2	6.8	7.5	9.0	6.3
Sulfaquinoxaline (SQX)	2.0 ± 0.2	9.2	8.1	4.0	9.1
Sulfamethoxazole (SMZ)	3.0 ± 0.3	8.6	8.5	6.5	4.8
Enrofloxacin (ENR)	1.1 ± 0.1	7.4	7.4	5.1	4.9
Streptomycin (STR)	19.0 ± 2.8	86.8	80.1	72.6	91.5

*The IC_{50} was calculated from the extraction buffer system. $n = 12$.
†The LOD (limit of detection, mean + 3SD, $n = 20$) value was calculated from negative sample (chicken muscle, chicken liver, pig muscle, and pig liver).

chickens reared in an experimental unit without antibiotics treatment were then injected intramuscularly with different antibiotics. Muscle samples were collected after 6 h and subjected to both the hapten-chip assay (Fig. 3*D*) and the UPLC–MS/MS assay. Table 5 shows that the antibody hapten-chip system provides quantitative values that are highly consistent with those determined by UPLC–MS/MS.

Stability of the Hapten-Chip System

To determine their stability, the hapten-chips and reagents were randomly assigned to either storage at 37°C for 7 days or storage at 4°C for up to 6 months. Their recovery and sensitivity levels for the detection of eight antibiotics in four artificial positive standard tissue samples were tested every day for the first group and every month for the second. The hapten-chips and reagents consistently showed recovery and sensitivity comparable with those that were freshly produced regardless of the storage conditions tested. Importantly, the specificity of the hapten-chip for the detection of the eight antibiotics did not change, as evidenced by the fact that no negative sample became false-positive. Apparently, the hapten-chips

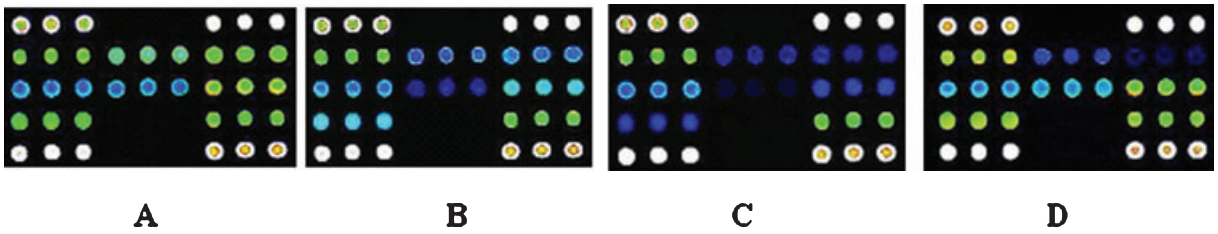


FIG. 3—*Representative chip analysis of the five drugs in chicken muscle samples. (A) Drug-free muscle sample. (B) Median concentration-artificial positive standard muscle sample (20 μg/kg for ENR, SM2, SMZ, and SQX and 150 μg/kg for STR). (C) High concentration artificial positive standard muscle sample (50 μg/kg for ENR, SM2, SMZ, and SQX and 250 μg/kg for STR). (D) SM2 and SMZ positive in incurred sample. ENR, enrofloxacin; SM2, sulfamethazine; SQX, sulfaquinoxaline; SMZ, sulfamethoxazole; STR, streptomycin.*

TABLE 4—*The precision and accuracy of analysis of tissue homogenates with added antibiotics by the antibody hapten-chip system.*

Drug	Fortified concentration (μg/kg)	Chicken liver		Pig liver		Chicken muscle		Pig muscle	
		RC (%)*	CV (%)†	RC (%)	CV (%)	RC (%)	CV (%)	RC (%)	CV (%)
ENR	20	88	10	94	12	99	13	87	15
	50	83	20	73	11	85	18	84	14
	100	78	20	72	8	77	13	74	20
SM2	20	94	17	77	21	81	12	83	17
	50	91	18	83	17	72	17	73	9
	100	73	19	82	16	76	17	91	7
SMZ	20	103	14	100	14	85	14	97	21
	50	97	17	85	12	95	8	91	19
	100	94	18	70	11	96	13	75	19
SIZ	20	91	19	87	20	89	14	78	10
	50	93	10	85	15	83	8	90	8
	100	82	11	82	9	86	9	87	13
SMM	40	108	17	92	16	75	10	86	14
	100	86	20	77	20	71	14	72	20
	200	72	18	84	8	73	16	84	15
SQX	20	85	20	83	16	80	14	77	16
	50	72	14	79	18	78	16	76	12
	100	70	21	76	20	75	13	78	15
STR	150	86	10	80	12	84	13	80	5
	250	80	12	87	17	79	19	80	11
	500	85	13	81	10	86	8	82	7
DSTR	150	76	15	101	17	81	12	103	16
	250	72	15	92	17	94	17	101	10
	500	86	12	94	18	92	14	88	17

ENR, enrofloxacin; SM2, sulfamethazine; SQX, sulfaquinoxaline; SMZ, sulfamethoxazole; STR, streptomycin; SIZ, sulfisoxazole; SMM, sulfamonomethoxine, DSTR, dihydrostreptomycin.

*Recoveries (RC, %) were calculated using the formula: calculated concentration of veterinary drug in the tissue sample/added concentration of drug in the tissue sample × 100.

†Coefficient of variation of the mean recovery (CV, %) was calculated as: (SD/mean) × 100. $n = 12$.

TABLE 5—*Comparison of the antibody hapten-chip system with UPLC–MS/MS for incurred chicken muscle samples.*

Chicken number	1		2		3		4	
Antibiotics	SM2	SMZ	ENR	SMM	SQX	STR	SIZ	DSTR
Hapten-chip*, μg/kg	44 ± 9	248 ± 25	236 ± 17	45 ± 9	94 ± 6	564 ± 13	107 ± 14	264 ± 28
UPLC–MS/MS, μg/kg	47	257	243	34	90	530	112	249

UPLC, ultra performance liquid chromatography; MS, mass spectrometry; ENR, enrofloxacin; SM2, sulfamethazine; SQX, sulfaquinoxaline; SMZ, sulfamethoxazole; STR, streptomycin; SIZ, sulfisoxazole; SMM, sulfamonomethoxine; DSTR, dihydrostreptomycin.

*Mean ± SD; $n = 3$.

and reagents can be stored at 37°C for at least 7 days or 4°C for at least 6 months without any losses in sensitivity and specificity.

Discussion

In the present study we describe a practical biochip assay system for the determination of the presence of antibiotic residues in different animal tissues. The system includes a simple but efficient multiresidue sample preparation procedure, fully controlled antibody hapten-chips, and dedicated software for subsequent data analysis. All these features make the system an attractive choice for different animal tissues compared with previous reports (12,13). The antibody hapten-chip assay was examined with four tissues from two different animal species. The analytical results with both

artificial positive standard samples and the incurred samples showed that the antibody hapten-chip system had a comparable accuracy and a similar sensitivity to the standard UPLC–MS/MS assay for the detection of eight commonly used veterinary drugs. Our method has several distinct advantages compared with the conventional methods used for residue analysis. First, the simple multiresidue sample extraction procedure generates good recovery for veterinary drug residues. Second, the hapten-chip can analyze at least eight different antibiotics with comparable sensitivity and specificity. Third, the high-throughput assay reduces the entire assay time from ordinarily 1 day or more to 3 h.

We anticipate that the application of the antibody hapten-chip system would be to provide a high-throughput method to simultaneously screen for the potential presence of multiple residues, providing a method to prioritize samples for conventional LC–MS/MS residue analysis. In combination with the rapid extraction procedure the chip system could provide an integrated vehicle for the accurate and high-throughput assay of many samples, as may be required at food processing plants and at food testing centers. We could conveniently handle six tissue samples and obtain data within 3 h with one technician.

The use of antibodies with high affinity for individual antibiotics realized an antibody hapten-chip system which has high specificity as well as exceptionally high sensitivity for antibiotic residue analysis. The LODs of the antibody hapten-chip system for all eight drugs were lower than required by both China and EU MRL provisions. This feature makes it a reliable screening method to avoid failure to detect contaminated samples. We anticipate that the ongoing development of antibodies with different specificities can be used to either increase the sensitivity of particular assays or to widen the range of detectible antibiotics.

Although the immunoassay tests indicated that the antibodies had very high specificity, we examined only a limited number of the possible antibiotic drugs that might be encountered in an industrial setting. The antibody hapten-chip system can therefore best serve as an early screening tool, as even such specific antibodies may be capable of cross-reacting with several related analytes with very similar core structures. Such limited cross-reactivity, however, can provide valuable information on the potential presence of a wider number of antibiotics. When a specific analytical method is not available for a new antibiotic drug, quantifiable cross-reactivity may be a useful means for initial detection, and the identity of the drug could then be confirmed by LC–MS/MS analysis.

Cross-reactivity is a double-edged sword. Currently, the system measures both the combined total of SMZ, SIZ, and SMM, and the combined total of STR and DSTR in the food sample but cannot identify individual compounds. This still satisfies the respective veterinary residue screening requirements. For example, sulfonamide MRLs in EU refer to the combined total residue of all SAs present, not to individual compounds within the grouping. ENR screening, however, is still an area that needs further improvement. In addition, the assay could detect ENR but not ciprofloxacin because the ENR antibody has little cross-reactivity with ciprofloxacin. An antibody showing suitable ciprofloxacin or generic fluoroquinolone cross-reactivity should be developed to give the sum of ENR and ciprofloxacin residues. Such an antibody would meet the EU MRL for ENR, which is the sum of ENR and ciprofloxacin.

In addition, the new multiresidue sample extraction method was also a key to the good performance of the assay system. The current extraction buffer had good resolution for each of the eight drugs. However, the greater the number of drugs that need to be analyzed, the more difficult it will become to develop a single utilitarian extraction procedure. The extraction method also showed good recovery of ciprofloxacin (data not shown) which also has declared MRLs. The present antibody hapten-chip system does not include a specific antibody for detection of ciprofloxacin, and its development is a current priority to widen the utility of the assay. Additional antibodies with specificities for a variety of veterinary antibiotics will be required for the detection of large numbers of different drugs that are commonly used by the animal production industries. Hence, ongoing development and testing of tissue extraction methods and the chip assay system will be required to introduce additional tests.

In principle, such a competitive immunoassay-based hapten-chip technology could be developed to detect a much greater number of low molecular weight substances, such as performance enhancing drugs used by athletes, and even a selection of environmental pollutant substances provided that appropriate high specificity antibodies and antigen conjugates are available. In this context, we have previously described the development of such hapten-chip technology for detection of banned substances such as anabolic steroids and doping agents (9–11). The design and development of a set of assay systems for such different purposes may be of benefit to detection agencies.

In conclusion, we have developed an effective analytical screening system which combines a straightforward multiresidue sample preparation approach with a state of the art assay system—the dedicated sample extraction apparatus, the antibody facilitated hapten-chip, the laser confocal scanner, and the data analysis software. The entire chip assay system has been designed to generate both qualitative and quantitative data with high specificity and high sensitivity, and which can readily be automated for situations requiring high throughput. The quantification of antibiotics present in tissue samples injected with antibiotics or in tissue homogenates in which known amounts of antibiotics were added by the antibody hapten-chip system was consistent with UPLC–MS/MS assays of the same samples.

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