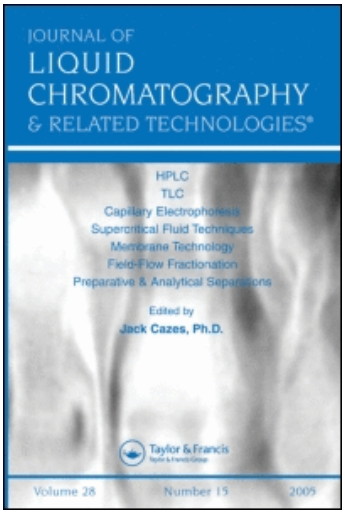

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TISSUE DRUG RESIDUE EXTRACTION AND MONITORING BY MATRIX SOLID PHASE DISPERSION (MSPD)-HPLC ANALYSIS

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ABSTRACT

The increasing implementation of immuno-, receptor and microbial inhibition screening tests for the monitoring of drug residues in foods of animal origin also requires that we be capable of confirming and validating the results of such tests in a timely manner. Methodology for the isolation and analysis of "detected" substances must be capable of performing rapid and efficient extractions that are amenable to instrumental determinations for the presence, level and, where possible, identity of the substance in question. We present here a summary of such methodolgy utilizing matrix solid phase dispersion (MSPD) as the isolation method and several simple isocratic HPLC/UV diode array and florescence methods developed for extracts so obtained for several drug classes as well as individual

drugs. The application of these methods for a variety of purposes in drug residue monitoring programs is discussed.

INTRODUCTION

The success of an analytical method for the isolation, identification, quantitation and/or confirmation of drug residues in tissues from food animals is determined by a number of interdependent factors. However, it is the method for extraction and isolation of the residues that can most directly influence the overall analytical result. In this regard, methodology for the isolation of drug residues from tissues has classically involved the extraction of 10-100g aliquots of sample by homogenization in several exchanges of organic or aqueous solvents. The combined extracts are then reduced in volume and/or processed through a series of back-extractions, pH adjustments and chromatographic purification steps. The intent is to isolate the drug residue with adequate (>60%) recovery, relatively free of interfering co-extractants that could complicate the analysis to be performed for the detection, identification or quantitation of the residue. This necessary specificity can make the method applicable to only one drug in a given matrix. Less specificity, however, often produces an unacceptably large quantity and number of various co-extractants. In the case of HPLC, this leads to an extremely complex chromatogram that requires the use of gradient elution programs to create an "analytical window" for the compound in question.

In general, this approach is time, labor and materials intensive and has too little application through a single method to other classes of drugs or even drugs closely related within a class. This approach will have to change if society is to keep pace with the increasing need to monitor more samples for more drugs. This need will require the development and application of screening technologies, such as immuno-, bacterial receptor or microbial inhibition assays for the detection of residues in foods of animal origin. However, these latter

assays must be supported by instrumental analysis techniques that accomplish a degree of simultaneous residue identification/ quantitation and/or confirmation for a number of compounds in a single analysis. Such determinative methods must also be rapid so that the advantages of conducting screening tests are not lost.

For the past several years our laboratory has been working to develop more generic procedures that would speed the process of screening animal derived foods for drugs. In this regard, a new extraction method, MSPD (matrix solid phase dispersion), was developed for the multiresidue/multiclass extraction of drugs from biological matrices, overcoming many of the difficulties associated with classical extraction techniques (1). This method involves the blending of a solid support (derivatized silica) and a tissue matrix to produce a nearly homogeneous dispersion of tissue cell membranes and matrix components. This semidry material can be packed into a column from which different drug residues can be eluted based on their solubilities in the polymer/tissue matrix. In this approach, a specific drug, a class of drugs or several classes of drugs can be rapidly isolated based on their distribution in the polymer/tissue matrix and polarity of the solvents used. The extracts so obtained often require no further processing prior to instrumental analysis.

We summarize here reports regarding the isolation of compounds in tissues using the MSPD technique for the extraction method and the development of simple, isocratic HPLC separation and diode array or florescence detection methods for identifying and quantifying many of these compounds. The possible implications of these results to food monitoring and residue analyses are discussed.

MATERIALS

Liquid chromatographic grade solvents of the highest purity available from commercial sources are used without further purification. The water for LC analyses is triple distilled and then passed through a Modulab Polisher I water purification system. Bulk C18 (40 μm, 18% load, endcapped; from Analytichem International,

Harbor City, CA, USA) is cleaned by making a column (50 ml syringe barrel) of the bulk C18 material (22 g) and sequentially washing with two column volumes each of hexane, methylene chloride, and methanol. The washed C18 is vacuum aspirated until dry. Ten ml syringe barrels thoroughly washed with double distilled water and air dried before use are used to prepare the MSPD columns.

METHODS

By adding 0.5 g of tissue (milk, kidney, liver, fat or muscle) to C18 or some other appropriate lipophilic polymer-derivatized silica column packing (2.0 g) in a glass or agate mortar and gently grinding the material for 30 seconds with a pestle, a nearly homogeneous mix of tissue components "dissolved" or dispersed into the solid phase packing material can be obtained. The homogeneous mix is transferred to a syringe barrel column. The resulting column may be eluted with a single solvent, two different solvents or a series of solvents in order to elute a drug, several drugs, or several classes of drugs from the column. Further purification or the use of co-columns prior to detection by immunoassay or instrumental analysis may be required according to the drug and/or matrix examined.

Generic extraction procedure:

Two grams of C18 are placed in a glass or agate mortar and a sample of tissue (0.5 g) or milk (0.5 ml) is placed directly onto the C18 packing material. For fortified samples, or for preparation of standard curves, standard drug solution is added to the tissue and the tissue is allowed to stand for 2 min prior to blending. The sample is gently blended into the C18 with a glass pestle until the mixture is homogeneous in appearance. The C18 packing material/tissue blend is placed into a 10 ml plastic syringe barrel that is plugged with a filter paper circle. The column head is covered

with a filter paper circle and the column contents are compressed to a final volume of 4.5 ml with a syringe plunger. A pipet tip (100 µl) is placed on the column outlet to increase elution time. Columns may be eluted by gravity flow or by use of a vacuum manifold.

This process is generic and has been applied to a wide range of compounds with varying degrees of modification. A summary of the extraction, clean-up and HPLC analytical procedures for neomycin, ivermectin, benzimidazoles, beta-lactams, chloramphenicol, chlorsulon, furazolidone, nicarbazin, sulfonamides, and tetracyclines in different sample matrices is reported below.

Extraction procedure for a specific type of drug:

Extraction procedure for neomycin: Bovine kidney is used as the tissue matrix. Bondesil, 40 µm, endcapped cyanopropyl silyl derivatized silica is used as the column material. The column is mounted on a vacuum manifold and three ml of hexane followed by 5 ml each of EtOAc, MeOH and MeOH:water (1:1) are added to wash the column. The column is eluted with 1 ml water followed by 8 ml of 0.1 N sulfuric acid. The eluate is collected and is derivatized for LC analysis using fluorescence detection (2).

Extraction procedure for ivermectin: Beef liver is the tissue matrix. Three ml of hexane, 6 ml of DCM: EtOAc (3:1) and 3 ml acetone are used successively to wash the column. The column is eluted with 6 ml methanol. The methanol eluate is evaporated to dryness and is reconstituted with derivatizing solution to perform LC analysis using fluorescence detection (3).

Extraction procedure for chloramphenicol: Milk is used as the tissue matrix. The prepared column is washed with 8 ml hexane and then 8 ml benzene. The compound is then eluted with 8 ml EtOAc. The solvent is evaporated to dryness and the sample is reconstituted with a solvent mixture (0.1 ml methanol and 0.4 ml 0.05N

phosphoric acid). The sample is filtered and analyzed by LC with a diode array detector (monitoring wavelength, 278 nm) (4).

Extraction procedure for chlorsulon: Milk is used as the sample matrix. The prepared column is washed with 3 ml hexane. The compound is then eluted with 3 ml ethyl ether. The extract is passed through a co-column of Florisil. The ether eluate is evaporated to dryness and the sample is reconstituted with 1 ml of solvent mixture (0.75 ml potassium phosphate buffer and 0.25 ml acetonitrile). The sample is filtered and analyzed by LC with a diode array detector (265 nm).

Extraction procedure for furazolidone: Milk (6), swine muscle (7), and chicken muscle (8) have been used as the sample matrices. The C18/matrix blend is used to prepare a column. The column is washed with 8 ml hexane. The furazolidone is then eluted with 8 ml DCM. The solvent is evaporated to dryness and the residue is reconstituted with a solvent mixture (0.1 ml methanol and 0.4 ml 0.17M phosphoric acid). The sample is filtered and analyzed by LC with a diode array detector (365 nm).

Extraction procedure for nicarbazin: Chicken muscle or liver is used as the tissue matrix. The C18/matrix blend column is washed with 8 ml hexane. The nicarbizin is then eluted with 6 ml acetonitrile. The solvent is evaporated to dryness and the sample is reconstituted with DMF. The dissolved residue is transferred to a Alumina B Sep Pak (Waters Corp.) column. After several column washes with hexane, the compound is eluted with 4 ml methanol. The sample is analyzed by LC with UV detection (365 nm). The confirmatory analysis is made using HPLC/MS (9).

Extraction procedure for sulfadimethoxine: Catfish muscle is used as the tissue matrix. The C18/matrix blend column is washed with 8 ml hexane. The sulfadimethoxine is then eluted with 8 ml DCM. The solvent is evaporated to dryness and the residue is reconstituted with a solvent mixture (acetonitrile: 0.05N phosphoric acid, 35:65).

The sample is filtered and analyzed by LC with a diode array detector (270 nm) (10).

Oxytetracycline: Catfish muscle is used as the sample matrix. The sample is blended with solid support material and 0.05g each of EDTA and oxalic acid. The resulting column was washed with 8 mL of hexane and the oxytetracycline was eluted with 8 mL of ACN:MeOH (1:1, v/v). The solvent was evaporated and the residue reconstituted with 0.5mL of LC mobile phase (see Table 1). The filtered sample is analyzed by LC with diode array detection (365 nm) (11).

Extraction procedure for a specific class of compounds:

Benzimidazoles: Swine muscle (12), bovine liver (13), and milk (14) have been used as tissue matrices. For swine muscle or bovine liver, the tissues are blended with octadecylsilyl derivatized silica packing material. The resulting column is washed with hexane. The benzimidazoles [thiabendazole (THI), oxfendazole (FBZSO), mebendazole (MEB), albendazole (ALB), and febendazole (FBZ)] are eluted with acetonitrile. The compounds are purified by passing the acetonitrile extract through an activated alumina column. The compounds are analyzed by LC analysis with UV detection. Extraction of seven benzimidazoles (THI, FBZSO, p-hydroxy-FBZ, FBZ-sulfone, MEB, ALB, FBZ) spiked into milk has also been performed. Hexane is used for washing the column. A solvent mixture (DCM:EtOAc, 1:2 (v/v)) is used for the elution of the drugs. No further purification is necessary. The compounds in the extracts are then analyzed by LC analysis with diode array detection (290 nm).

Sulfonamides: Milk (15), infant formula (16) and swine muscle (17) have been used as sample matrices. The resulting column is washed with 8 ml of hexane. Sulfonamides [sulfanilamide (SNL), sulfathiazole (STZ), sulfadiazine (SDZ), sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethoxazole, sulfisoxazole, and sulfadimethoxine] are eluted with 8 ml DCM. The DCM extract is

Table 1. Summary of drugs, matrices examined and LC conditions for analysis using MSPD as the extraction technique.

DRUG	MATRIX	SOLVENT SYSTEM	RUN TIME	COLUMN
Sulfonamides	milk (15)	90:10 0.05N phosphoric acid:ACN 1.0mL/min-5min, 2mL/min	16min	Supelcosil LC18, 3um 7.5cm X 4mm, 45C
	infant formula (16)	70:30 0.05N phosphoric acid:ACN 1.0mL/min	16min	Varian MCH10, ODS, 10um 30cm X 4mm, 40C
	swine muscle (17)	"	12min	"
Sulfadimethoxine	catfish muscle (10)	65:35 0.05N phosphoric acid:ACN 1mL/min	10min	"
Benzimidazoles	swine muscle (12)	60:40 0.05N phosphoric acid:ACN 1.0mL/min	16min	" 45C
	bovine liver (13)	"	"	"
	milk (14)	"	"	"
	bovine muscle (1)	67:33 0.05N phosphoric acid:ACN 0.75mL/min	25min	" 35C
Furazolidone	swine muscle (7)	60:40 0.05N phosphoric acid:ACN 1.0mL/min	8min	"

	chicken muscle (8)	70:30 0.05N phosphoric acid:ACN 1.0mL/min	6min	"
	milk (6)	"	8min	"
Chloramphenicol	milk (4)	65:35 0.05N phosphoric acid:ACN 1.0mL/min	8min	"
Beta-Lactams	bovine muscle (1)	80:20 0.05N phosphoric acid:ACN 1.0mL/min	10min	" 45C
Tetracyclines	milk (18)	70:30 0.01M oxalic acid:ACN 1.0mL/min	8min	" 40C
Oxytetracycline	catfish muscle (11)	70:27.5:2.5 0.02M oxalic acid: ACN:MeOH 1.0mL/min	6min	"
Ivermectin	bovine liver (3)	95:5 MeOH:water 2.0mL/min	20min	Econosil ODS, 5um 25cm X 4.6mm
Chlorsulon	milk (5)	3:1 pH7 0.01M NaH2PO4:ACN 2.0mL/min	10min	Econospere ODS, 3um 15cm X 4.6mm
Nicabazin	chicken muscle, liver (9)	75:25 MeOH:water 1.0mL/min	8min	
Neomycin	bovine kidney (2)	0.01M 1-pentanesulfonate and 0.1%acetic acid with 1.5%MeOH and 0.056M Na2SO4	20min	Supelcosil LC18 DB, 5um 15cm X 4.6mm

evaporated to dryness and is reconstituted with 0.1 ml methanol and 0.4 ml 0.017M phosphoric acid. The filtered sample is analyzed by LC with diode array detection (270 nm).

Tetracyclines: Milk is used as the sample matrix. The milk is blended with octadecylsilyl derivatized silica packing material containing 0.05 g each of oxalic acid and disodium ethylenediamine tetraacetate (EDTA). The resulting column is washed with 8 ml of hexane. The column is eluted with 8 ml of solvent mixture [EtOAc:ACN, 1:3 (v/v)] to obtain the tetracyclines (tetracycline, oxytetracycline, and chlortetracycline). The solvent extract is evaporated to dryness and is reconstituted with 0.5 ml solvent mixture (0.01M oxalic acid:ACN, 7:3). The filtered sample is analyzed by LC with UV detection (365 nm) (18).

Extraction procedure for multidrug residue from a single tissue sample:

For organophosphates (phenthion, crufomate, coumaphos, and famphur), benzimidazoles (FBZ, FBZSO, $FBZSO_2$, p-hydroxy-FBZ, FBZOH, MEB, THI, ALB), and beta lactams (penicillin, ampicillin, and cephapirin) in a single sample (1). Bovine muscle is used as the tissue matrix. A series of solvents are used to elute the different classes of compounds. Hexane, benzene, EtOAc, and methanol are used successively. All the solvent fractions are evaporated to dryness. Hexane and benzene fractions are reconstituted with 500 µl hexane and analyzed by GC with nitrogen/phosphorus detection for organophosphates. The EtOAc fraction is reconstituted and analyzed by LC using photodiode array detection, for the benzimidazole class of compounds. The methanol fraction is reconstituted and LC analysis is performed for beta lactams using diode array detection.

HPLC Analyses:

The columns, solvent systems and conditions employed in assaying the extracts obtained as described above are given in Table

1. All analyses, except as noted, were conducted using a Hewlett Packard 1090M HPLC/diode array/Chemstation system equipped with an autosampler and a column oven. Injections were automated and were from 10-20uL from a final sample volume of 0.5-1.0mL for the various assays.

RESULTS

The extraction methods summarized provided recoveries of greater that 60% for the individual compounds over the range of concentrations examined. All of the methods gave correlation coefficients for linearity of 0.99 or better, whether for a single compound or a class of compounds. The limits of detection obtained for these procedures were at or below the action levels established for the various drugs by the different regulatory agencies at the time. In most cases no clean-up steps were required post-elution and concentration. The extracts, analyzed as described in Table 1, were relatively free of contaminating co-extractants, allowing the drugs to be assayed using simple isocratic solvent systems, with moderate flow rates (1-2mL/min) and with relatively short total run times (6-25min).

DISCUSSION

Drug residue monitoring programs rely on the development of analytical techniques in order to regulate and to guage the nature and severity of any drug contamination of the food supply. This methods development and the subsequent implementation of such methods is not a trivial task. As in the case of most analytical procedures, there are a number of factors to be considered in developing methods that not only perform at a given analytical level but that are also appropriate for the task at hand. The present requirement is to develop new methodology to assay more food animal products for more drugs and pollutants and to do so in a

timely manner, preventing entry of the contaminated items into the market before the analytical result is obtained.

Analytical methodologies for drug residues in tissues from food animals have been developed from a number of sources. These methods may be employed for drug screening, determination of the residue identity and level and/or for confirmation of the structure of the residue. Many of these methods are "official" or validated methods that have undergone interlaboratory examination and are of excellent analytical quality. However, these individual methods are most often designed for a specific drug or metabolite, isolated from a specific matrix, and assayed by a specific LC, GC or other chromatographic technique that may, unfortunately, have little or no application to other similar drugs or sample matrices. This has led to a methods morass and the need to develop a new method for each new drug in each of several possible matrices. The complexity of many of these methods also make them impossible to apply to a statistically significant number of samples. For this and other reasons such an approach has made attempts to test more samples for more drugs of concern virtually impossible, both in terms of overall cost and total response time. Such an approach cannot possibly keep pace with the ever-increasing numbers of veterinary drugs available and their increased use in expanding food animal production and health practices around the world.

Solutions to this problem have been slow in coming but are nevertheless being recognized and implemented throughout the industry. This is especially true for milk. In terms of screening capability there has been an increased availability and use of microbial inhibition, immuno- and bacterial receptor assays for the detection of, primarily, antibiotics and antimicrobials in milk and milk products. This approach to drug testing, wherein samples are rapidly screened by drug-class or individual drug specific immunoassays to distinguish those samples that are drug free from those that are suspicious or of concern, is more in keeping with that frequently applied today to forensic, drugs of abuse and clinical/emergency room samples. However, in these instances one may often directly assay milk, blood or urine without the requirement of having to perform an extraction or drug isolation

procedure. Thus, large numbers of samples can be rapidly processed and assayed and the results rapidly obtained. Only those samples giving a clear indication of drug presence are usually taken for more thorough analysis. This is not the case, however, with drug residues in tissues. The residue must be removed from the tissue matrix prior to performing screening tests, such as microbial inhibition, receptor or immuno-assays. However, this difficulty may yield to extraction protocols similar to those presented here. We have observed that MSPD in general can be used to rapidly isolate a range of residues from a single or several tissue samples and that the extracts obtained can be used for immuno- and/or bacterial receptor assay screening (Barker, et al., In Preparation 1992).

As immunoassay and other technologies for the rapid screening of milk and tissue samples are developed and applied there will be an increasing need for the ability to rapidly assess and confirm the results of presumptive positives obtained from such assays. To simply accept the result of an immuno-, receptor or microbial inhibition assay as definitive proof of the presence of a drug or as indicative of a violation is analytically unacceptable. Such assays must be supported by extraction and instrumental analyses that validate the presence of a drug, determine the identity of a drug and the level of a drug before accepting the result. It is highly probable that there are many possible variations in sample matrices, cumulative drug effects, cross-reactive drugs and non-drug species effects, disease states, as well as many other well or ill defined interferences that can lead to false positive results from receptor or immunoassay kits. The use of screening tests alone to refuse or destroy product and bring into question the producer's practices or reputation is scientifically and, perhaps, legally and morally indefensible, given the fact that false positive results from such tests will occur. Therefore, methodology that is applicable to the various food animal matrices that can isolate and identify the drugs of concern and provide a rapid process for definition of screening assay response is urgently needed.

We propose that the results presented for the extraction and analysis of a wide range of compounds from various matrices using MSPD/HPLC provide a starting point for finding solutions to some of

these aspects of the problem. The MSPD processes, as described, can rapidly provide extracts that can be screened by various assay formats or that can be analyzed by simple isocratic HPLC methods. This characteristic makes the overall approach applicable to the rapid fractionation, isolation and instrumental analysis of suspect samples, as determined by rapid screening tests. Individual drug or drug class analyses can be developed that are free of many interfering co-extractants and that do not require time and solvent-consuming gradient elutions to create an analytical window for detection or determination of the various residues. This simplifies and speeds the determinative process and could be more readily applied to multiple suspect samples for multiple and various drugs on a more routine basis. Indeed, for most of the methods developed in our laboratories (1, 4, 6-8, 10-18) we have found that a simple isocratic solvent system consisting of 0.05N H3PO4: ACN over a range of 60:40 to 90:10 (v/v) provides adequate resolution of the majority of compounds examined. These results are directly linked to the cleanliness of the extracts and makes it feasible to simplify the overall process of HPLC quantitation/ identification/ screening method validation in a residue monitoring laboratory.

The use of a combined HPLC/UV diode array system in such assays provides an ability to determine, with a degree of certainty, the identity of the residue examined and that of possible contaminants. The retention time of a drug, validated against standards and blanks, and a complete UV spectrum, correlated by correct wavelength maxima /minima, do provide a degree of identification/confirmation certainty that will, in many cases, suffice in making a judgement. Thus, coupled with its ability to quantitate, such a system can provide data to address the questions raised by the result from either an immunoassay, receptor or inhibition test: What drug is present? How much is present? Is there a violation. As more data become available concerning the factors that can lead to false positive results on screening assays for foods the need for methodology to quickly provide these answers will become more evident and urgent.

However, diode array is often inadequately sensitive for the detection of various drug residues and there is a limit wherein one

can obtain useful UV spectra. This is to a large measure dependent on the sensitivity of the equipment used and the structure of the molecule being assayed. Many compounds require florescent, UV or electrochemically active tagging for detection. It should be noted that when one needs to conduct such derivatizations the analytical result obtained is highly dependent on the number of reacting co-extractants. Although complete spectra for "validation" cannot be readily obtained by these methods the use of a retention time and the presence of a compound at that time giving correct responses to one or more specific detectors would greatly assist, in most cases, in answering the aforementioned questions.

A different approach would be to isolate the HPLC effluent and detect the presence or absence of a reacting substance by running an aliquot in the original screening test, using the immunoassay, receptor or inhibition test as the detector. This approach may have a higher degree of uncertainty since a cross-reacting substance could still be isolated by the same procedure and produce a further false-positive result. This approach essentially uses the screening method to validate itself and should be considered unreliable and of little absolute value. However, providing the ability to validate low nanogram/g levels of some drugs considered to be violative at this concentration and that are detectable by sensitive immuno- or receptor assays may prove to be a major challenge. Fortunately, most drugs of concern are violative at levels readily detectable by the methods given here as well as others.

Nevertheless, in the case of methods using MSPD, sample size and limits of detection are of concern. However, the limits of detection of analyses conducted to validate assays will be affected by a number of interrelated factors; 1) Sample size, 2) efficiency of the extraction/isolation method, 3) final sample extract volume, 4) amount of extract injected for analysis, 5) sensitivity and specificity of the detector(s) utilized and the ability of the compounds to respond to them and 6) the absence of interfering substances. A further complication is that some class-specific screening assays may possibly respond to the presence of several drugs within a class (sulfonamides, for example) that, taken individually, fall below a method's ability to detect and identify. The MSPD process utilizes

relatively small sample sizes (0.1-1.0g in some applications) which limits the total number of molecules ultimately available for detection. This small sample size also provides a degree of simplicity to the method and plays a major role in the speed with which it can be performed and the cleanliness of the extracts obtained. As an extraction technique for subsequent use in screening methods this small sample size will not, in most cases, be a complication. However, for some instrumental analyses too little drug may be extracted to attain a necessary limit of detection. One example is the detection of sulfonamides in milk, with a need to detect and subsequently validate the presence of drug to as low as 5.0ng/mL in the United States. The method presented here, using 0.5mL of milk, had a limit of detection of 31.2ng/mL, well below the violative level of 100ng/mL established at the time of development. However, it should be noted that this assay, as in the case of most of those reported from our laboratory, involved the injection of relatively small sample sizes (10-20uL) from a final extract volume of 0.5-1.0mL. Further, the samples were assayed on large bore/particle size LC columns (30cm X 4.6mm, 10um particle size) with detection being conducted using older, less sensitive diode array technology. We have observed that injection of 100uL from a final sample volume of 250uL onto a narrow bore 5um particle size column, with detection by a variable wavelength UV detector (270nm) provides a limit of detection of 4ng/mL for milk samples. This optimization for sensitivity was also accompanied by an increase in the background, as would be expected. However, the extracts are relatively free of any interferences and this enhancement can be performed without effecting specificity and resolution of the components (Unpublished observations). The variables listed, exclusive of sample size, are more definitive of detection capability in many cases. Although translatable as a value, it is the number of nanograms-on-column that are being detected that establishes the final ng/g of sample limit of detection determined. The ability to use a clean extract, allowing injection of a larger portion of the total sample and clear detection by a specific and sensitive detection system can provide, in most cases, adequate detection while taking advantage of the savings allotted from conducting a microscale extraction procedure. Thus, small

sample size need not be a deterrent to attaining low nanogram levels of many drugs.

The techniques presented may prove applicable on several levels for performing residue monitoring; rapid isolations or preparation steps for samples prior to immuno-, receptor or microbial inhibition assay screening, rapid extraction for determination of the nature of a screening test response, and extractions for the final determinative steps. MSPD greatly reduces the degree of sample manipulation, solvent usage and disposal, technician time and the subsequent time to obtain results for a given analysis. the cleanliness of the extracts allows rapid, isocratic LC analyses to be performed that can provide a degree of validation of a screening test result as well as quantitation and identification of the residue. MSPD is a generic approach for the disruption and extraction of biological samples that is also amenable to automation. MSPD and the LC techniques presented will not be applicable in every case to be encountered in the field of drug and pollutant residue monitoring. However, we do propose that they may serve as a major adjunct to existing analytical procedures and that they offer an enhanced capability to perform the most difficult aspect of drug residue analysis; the isolation of the target compounds from the sample matrix.

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