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EQUINE FORENSIC SCIENCE:

AN OVERVIEW OF THE SCIENTIFIC METHODS

Prepared by Members of

THE TESTING INTEGRITY PROGRAM (TIP)

for

THE NATIONAL THOROUGHBRED RACING ASSOCIATION

October 28, 1998

UK

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ACKNOWLEDGEMENTS

This outline was prepared by several members of the Testing Integrity Program (TIP), with specific input from Drs. Cynthia Kolias-Baker, Scott Stanley, Chris Nattrass, Fritz Lehner, Tom Tobin, Thomas Wood, David Tiffany and Wyndee Carter.

When reviewing this outline, one must remember that the testing strategies implemented by individual laboratories vary, depending on the resources available to the laboratory, the educational background, scientific strengths and inclinations of the laboratory staff, and the regulatory requirements and governing regulations of the controlling authority. There is, therefore, at this time no consensus as to how equine testing should be performed. As such, individual members of TIP may and do vary their scientific approaches depending on specific circumstances.

One of the most important aspects of a Quality Assurance Program is to foster good communications between laboratories. Good communications and dialog between laboratories will unify the resources and diversity of the various laboratories. Diversity in approaches to a common problem are not a disadvantage, but can be used to an organization's advantage when a proper dialog is established.

Finally, to enable approximate quantitative comparisons between screening methods, we have arbitrarily chosen a sample load of 20,000 urine samples/year for our hypothetical laboratory.

EXECUTIVE SUMMARY

Equine drug testing consists of a high throughput* "screening" process followed by a much lower throughput "confirmation" process. The laboratory first rapidly screens all suitable submissions for preliminary evidence of improper substances. Screening methods include **ELISA** (Enzyme Linked ImmunoSorbent Assay) screening, **TLC** (Thin Layer Chromatography) screening and, more recently, **Instrumental** screening.

The **ideal screening** method is sufficiently sensitive and specific and has **high throughput**. By definition, a screening "positive" simply suggests the presence of a drug; unequivocal demonstration of its presence requires the use of mass spectral confirmation or other appropriate confirmatory methods.

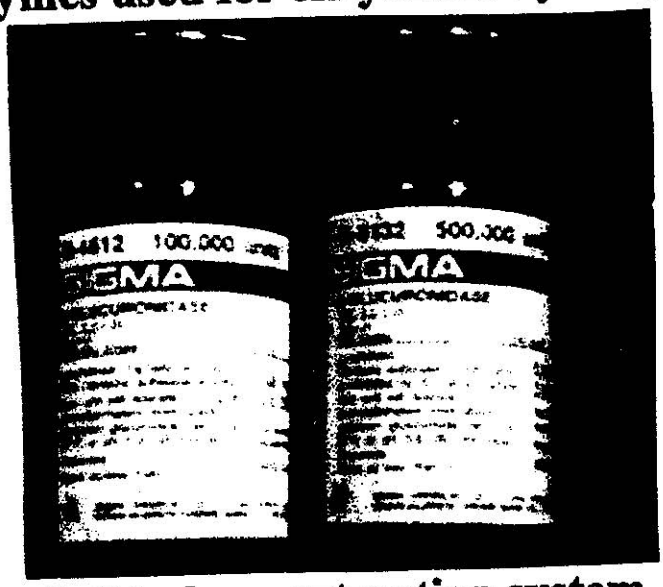
Effective screens are the core of equine drug testing; without high quality information generated by the screening methods, useful potential identifications are not fed into the confirmation process.

Mass Spectral Confirmation, performed under rigorously controlled conditions, can specifically determine the molecular weight of the drug and break it into a specific fragmentation pattern of characteristic parts. A properly obtained Mass Spectrum usually constitutes definitive identification ("a fingerprint") of a drug/analyte. All that generally remains is independent confirmation of the chemical findings.

Independent or "split sample" confirmation of mass spectral findings is provided by submitting a "split" sample to a referee laboratory. Independent confirmation verifies and consolidates the chemical evidence and the matter then proceeds to regulatory review.

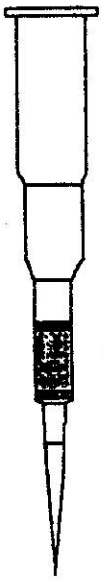
*Throughput: The rate at which samples can be processed for useful information.

Enzymes used for enzymatic hydrolysis.



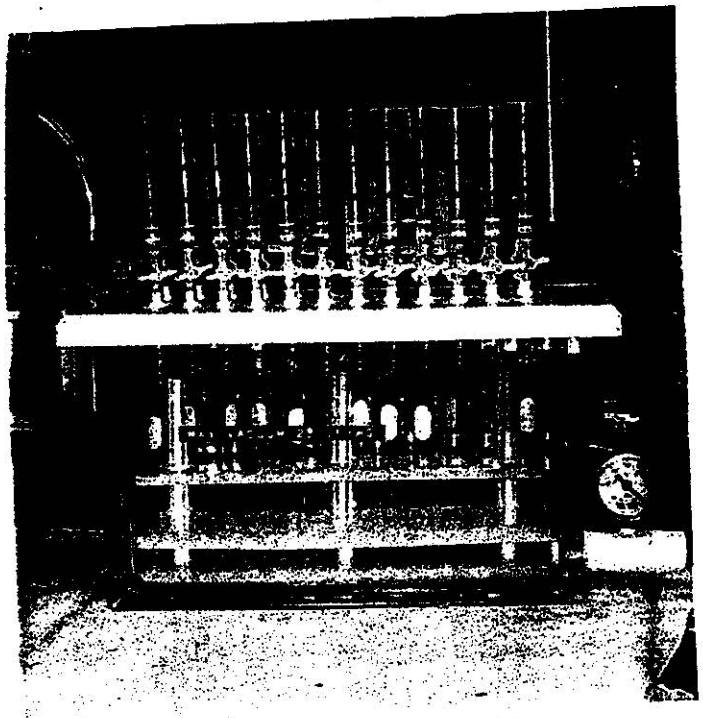
Solid phase extraction system

Individual/Solid phase tube



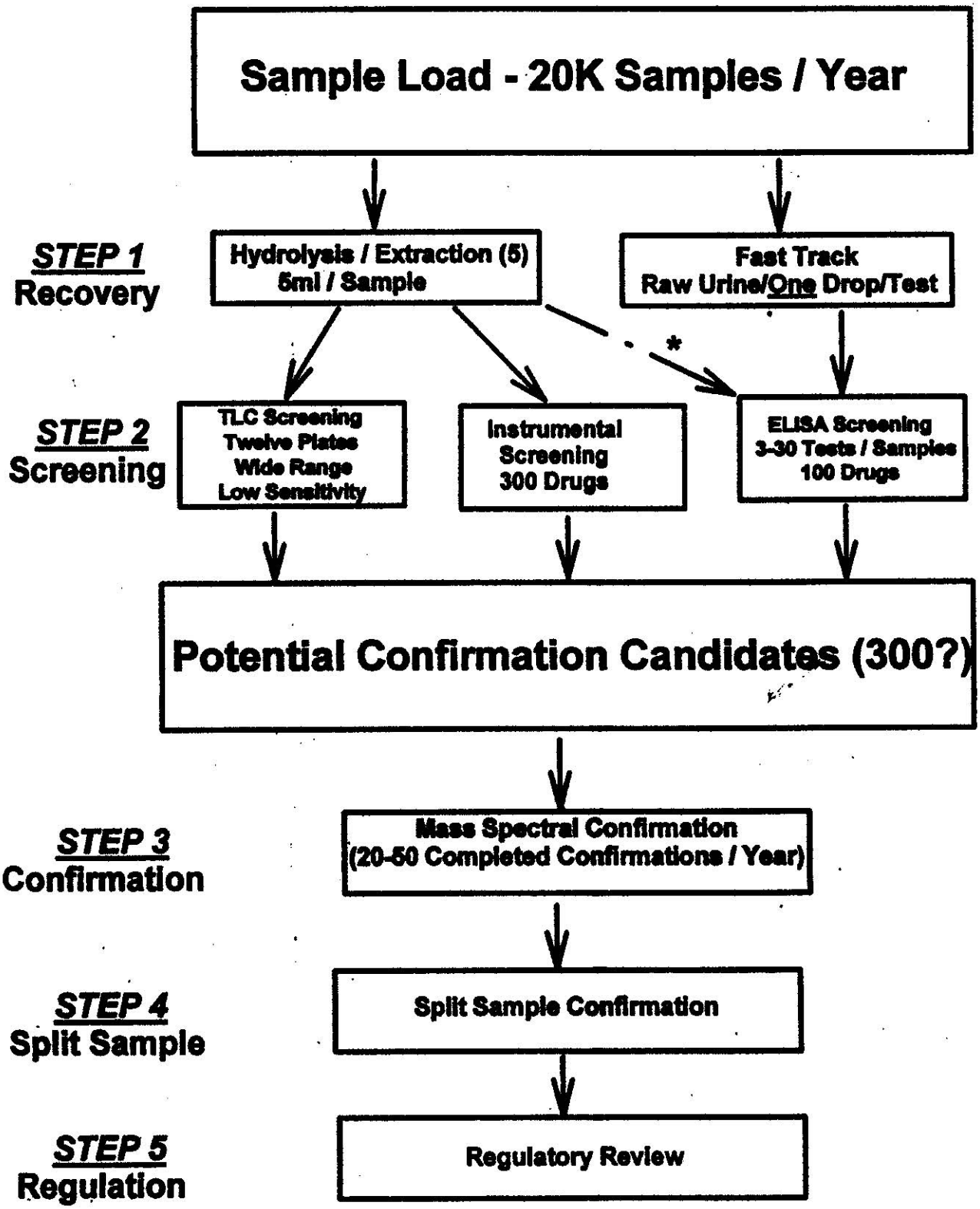
50 fold concentration of sample.

Array of tubes

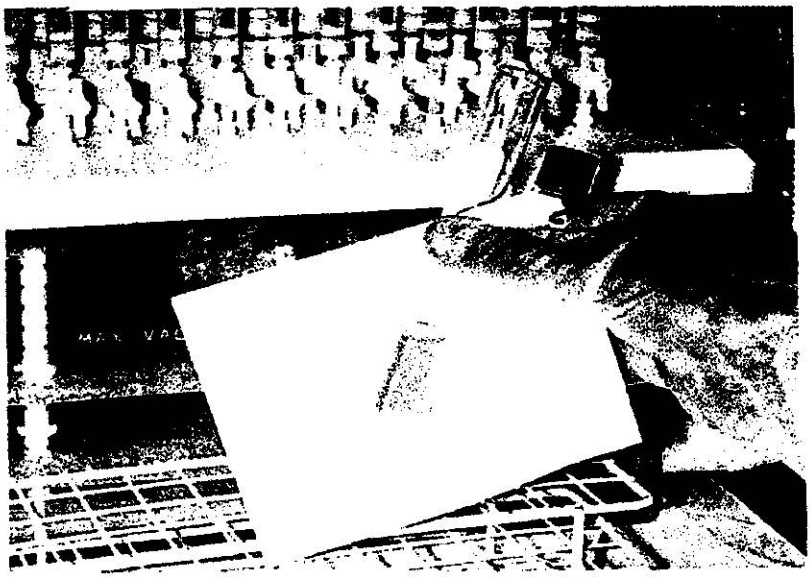


Solid phase extraction manifold fitted with World Wide Monitoring drug screening extraction tubes.

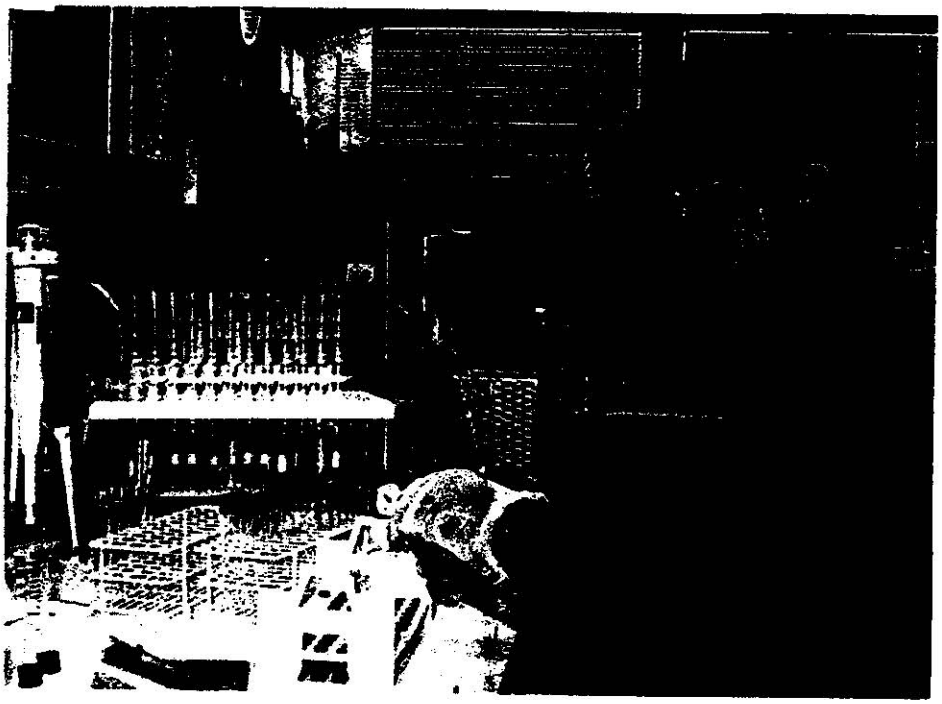
Flow Chart of Equine Drug Testing



* Use of a concentrated extract for ELISA screening increases its sensitivity, as has been shown for clenbuterol.



Relative volumes of urine (left) and extracted concentrated urine (right).



Preparation of extracted derivatized equine urine samples for GC/MS quantification analysis.

SEQUENCE OF TESTING / REGULATORY EVENTS

- (COLLECTION OF SAMPLE.)

1) **STEP ONE:** Extraction/Recovery/Isolation/ of Drug/Metabolite from Sample:
(Page 9)

2) **STEP TWO:** "Screening" of samples for evidence of the presence of prohibited substances:

Screening methods:
A: Thin layer chromatography (Page 11-13)
B: ELISA testing (Page 14-17)
C: Instrumental screening (Page 19-19)

↓

3) **STEP THREE:** Suspect samples go from screening to CONFIRMATION ANALYSIS. A high quality mass spectrum usually constitutes definitive evidence of the identity of a drug / metabolite.
(Page 20-23)

↓

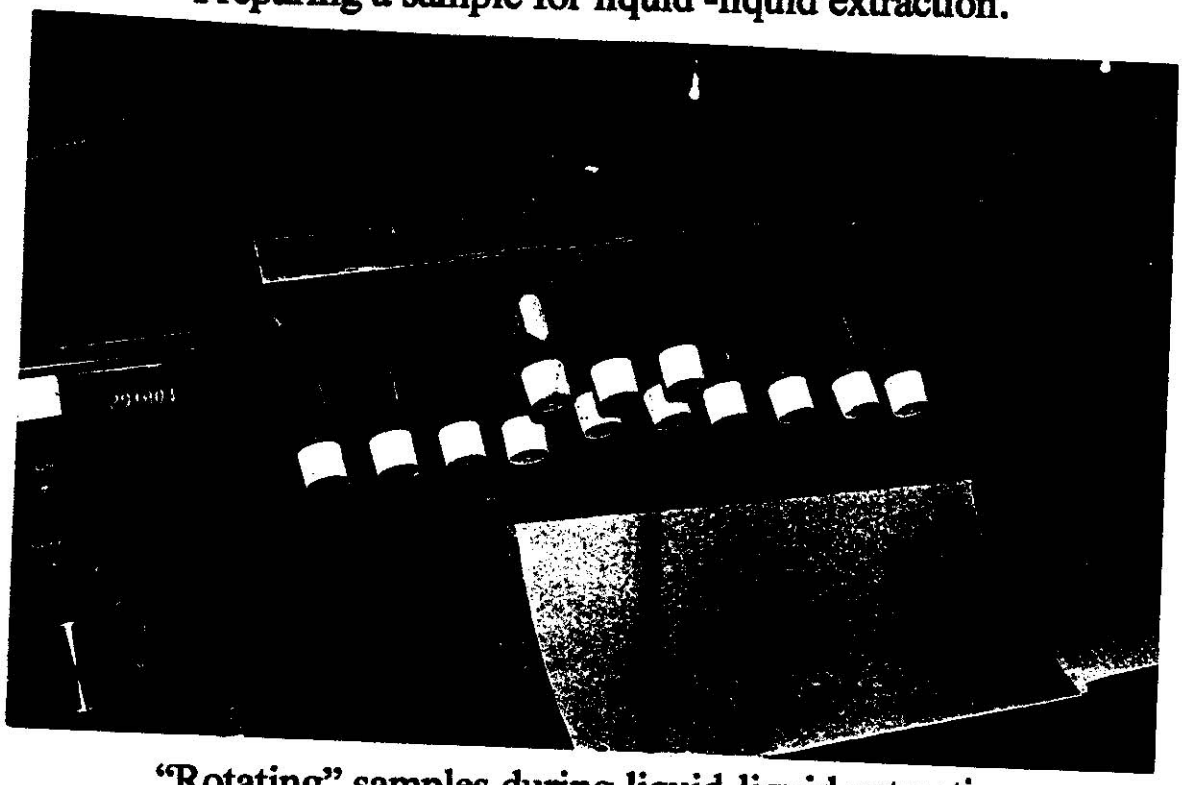
4) **STEP FOUR: "Referee"** sample confirmation: The governing regulations in some jurisdictions allow for a repeat analysis of a "split" or "referee" sample at second laboratory selected by the trainer from a list of approved laboratories. Once the identification of the analyte in the referee sample has been independently confirmed the chemical data are unlikely to be contested.
(Page 25)

↓

5) **STEP FIVE: Regulatory review**: ARCI classification / residues of therapeutic medications / standardized testing / mitigating factors / penalty.
(Page 25)

DRUG EXTRACTION/RECOVERY/ISOLATION

Preparing a sample for liquid -liquid extraction.



"Rotating" samples during liquid-liquid extraction.

STEP ONE: DRUG RECOVERY: Hydrolysis /Extraction / Screening / Derivatization.

For most analytical techniques (except ELISA*) drugs / metabolites must be:

1) **HYDROLYZED** from the glucuronide / sulfate moieties that horses link many drugs to for excretion. Hydrolysis may be enzymatic, acidic or alkaline based, depending on the drug/analyte.

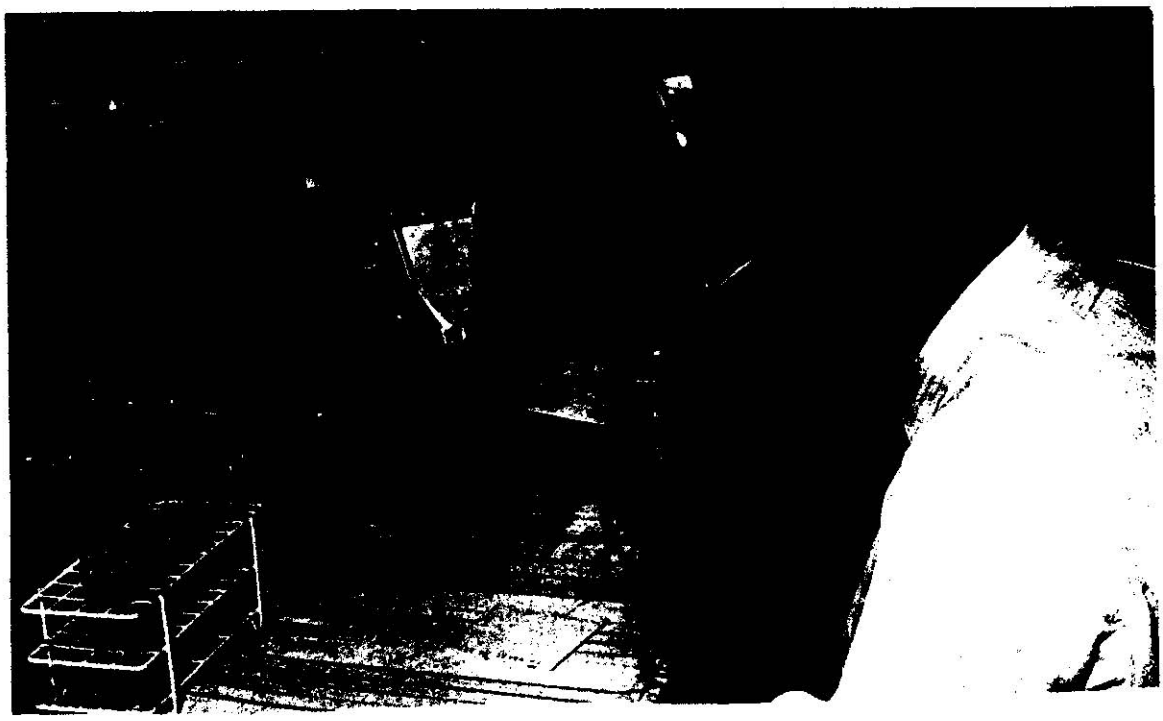
2) **EXTRACTED / RECOVERED:** drugs or hydrolyzed drug metabolites are isolated by **"Solid Phase"** or **"Liquid-Liquid" extraction.** These steps yield a **concentrated** extract of the drug / metabolite in question.

3) **SCREENING:** This **concentrated** extract goes to preliminary screening by the three listed methods. If evidence of a prohibited substance develops, it generally goes to mass spectral confirmation.

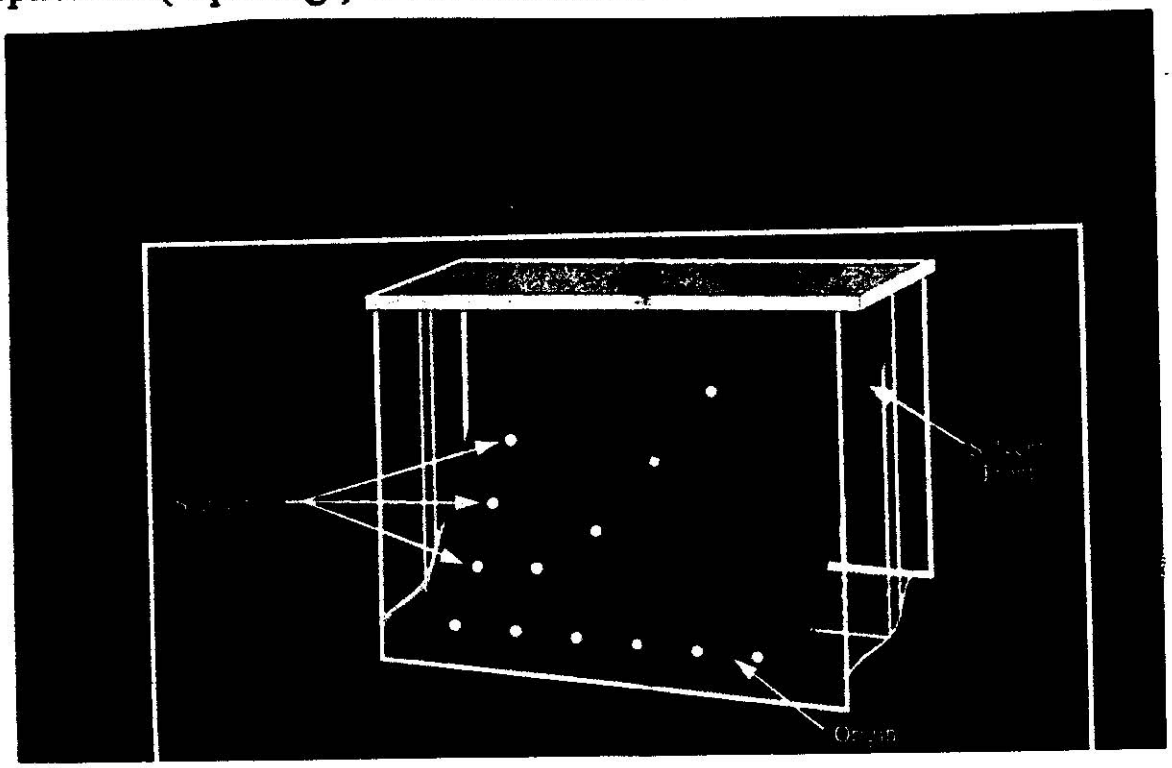
4) **DERIVATIZATION:** For Mass Spectral confirmation the isolated drug/ metabolite may need to be chemically modified (derivatized). This is especially so for **Gas Chromatographic-Mass Spectral** work, where derivatization may be necessary to make the drug/analyte **volatile** and thus amenable to analysis.

* **ELISA** screening is generally performed on a single drop of unprocessed (raw) urine. However, if an extraction (concentration) step is carried out first, then the lower limit of detection (sensitivity) of the ELISA test can be significantly increased. (See dashed arrow, steps 1-2, page5)

SCREENING BY THIN LAYER CHROMATOGRAPHY



Application ("Spotting") of the concentrated urine "Extract" on a TLC plate.



Running a TLC plate: The solvent (dark blue) moves up the plate, taking the drug "spots" varying distances up the plate.

STEP TWO (A): SCREENING BY THIN LAYER CHROMATOGRAPHY (TLC).

1) TLC screening utilizes postcard sized glass plates covered with a **"THIN LAYER"** of silica gel, 1/32 inch thick.

2) The analyst **Applies** ("spots") a small volume of **concentrated extract** of urine to the TLC plate: one plate will run 10-20 individual samples.

3) The analyst **Runs** a plate by placing it in a TLC tank and letting solvent migrate up the plate by capillary forces.

4) The analyst **Develops** a plate by drying and overspraying or dipping it in color inducing solutions. **("CHROMATOGRAPHY").**

5) The analyst **Reads** a plate, generally by eye, sometimes under ultraviolet light, looking for suspicious spots.

THROUGHPUT

Throughput: High potential. Multiple **batched** TLC systems can be used, along with several oversprays. Estimated # of tests at five to ten times sample load, or 100–200K tests on 20K per annum sample load.

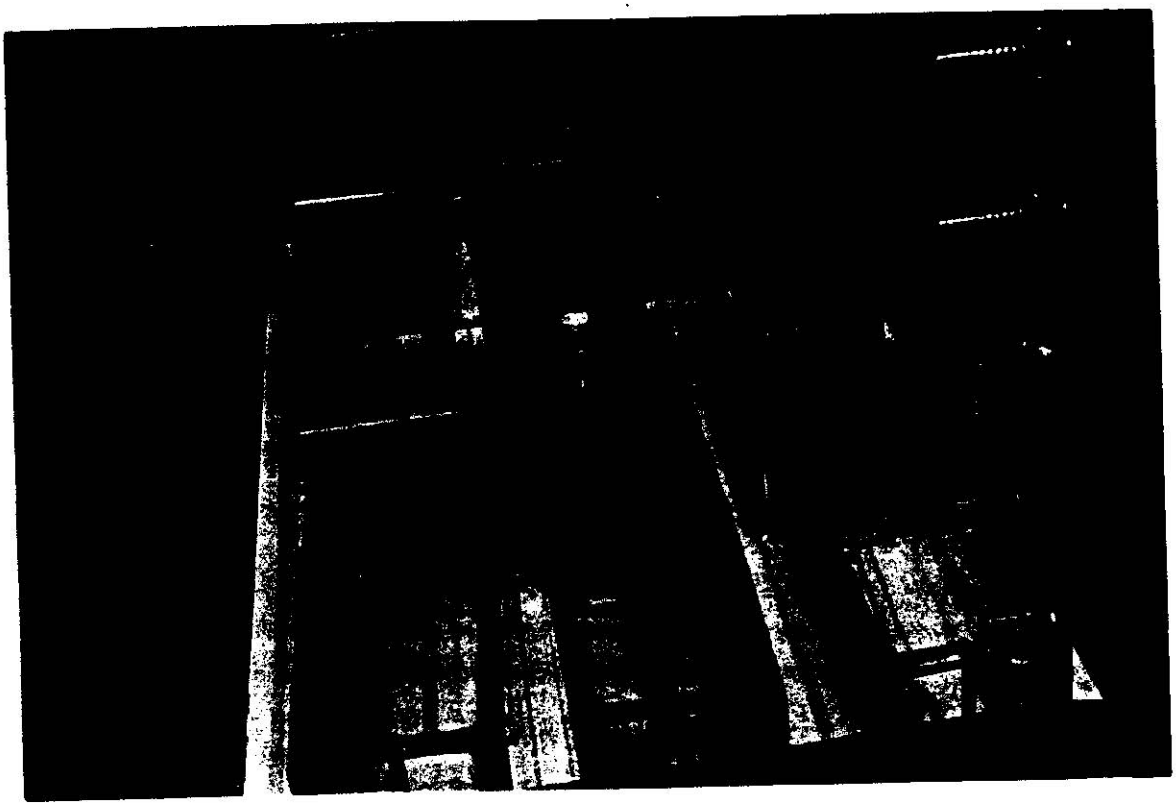
ADVANTAGES

1) Economical: TLC components are inexpensive, however, the overall process is labor intensive and not readily automatable.

2) Rapid: The process takes approximately 4-8 hours and the **throughput** is relatively high because samples can be **batched.**

3) Broad spectrum: TLC covers a wide range of drugs/analytes: **completely unexpected, previously unidentified analytes may appear** as a new "spot" on the plate. However, reading TLC plates requires experience and is somewhat of an art form. Also, "chasing" "new" TLC spots is generally unrewarding. These demands are typically placed on one individual.

4) The system is simple and very easy to modify and adapt.



An Array of TLC tanks for batch analysis.

"Spraying" a TLC plate for development.

DISADVANTAGES

1) Not a particularly sensitive technique; generally not effective below 100 ng/ml. Cannot be used to detect many of the most potent drugs.

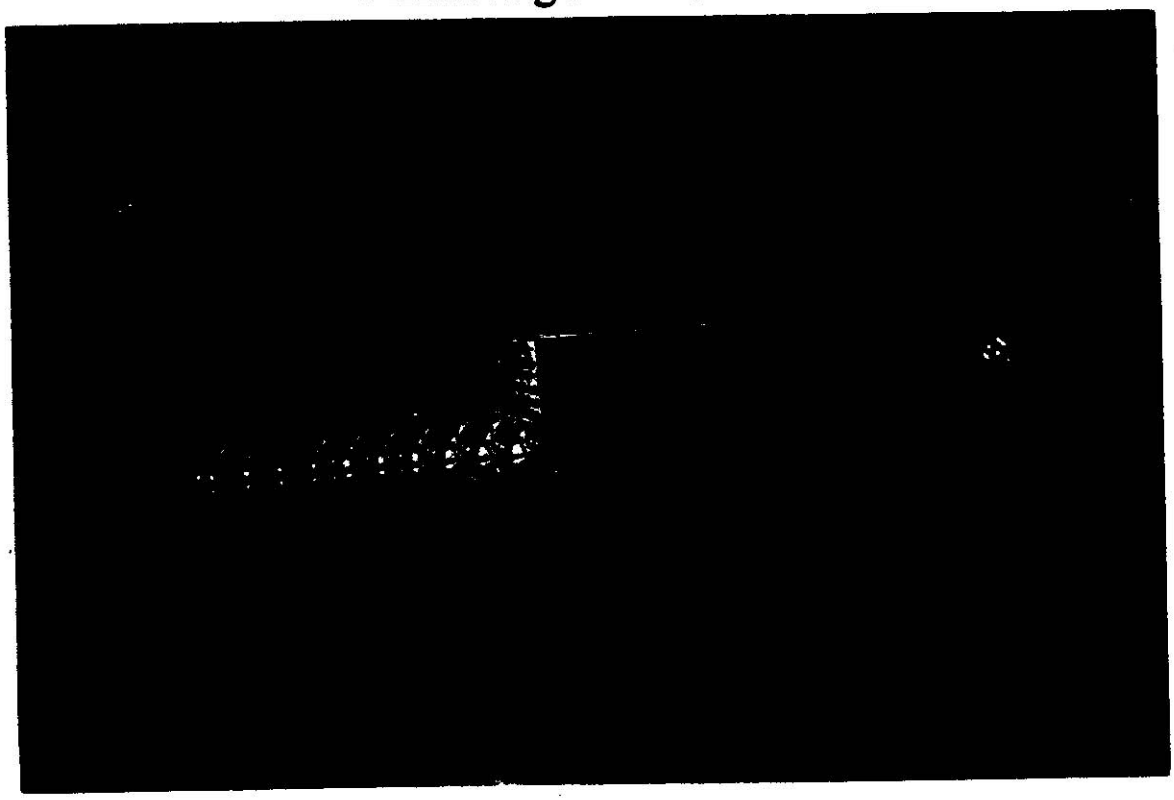
2) Use requires experienced, dedicated operators; loss of such operators can dramatically affect detection capability.

3) Possible health risks from oversprays / solvents.

4) Labor costs much higher than those for ELISA; also costs of solvent purchase and disposal.

Dr. Jerry Blake reading TLC plates:

Performing an ELISA test:



Reading an ELISA: Blue indicates "negative", and white indicates "positive".

STEP TWO (B): SCREENING BY ELISA.

1) ELISA= Enzyme Linked ImmunoSorbent Assay.

2) Antibody based tests: highly sensitive, routine sensitivity of about 10 ng/ml (parts/billion), may be increased to 100 parts/trillion (0.10 ng/ml) if specific drugs/analytes such as clenbuterol are extracted and concentrated first. (See again, dashed arrow, steps 1-2, page5)

3) Works similarly to home pregnancy tests: **simple** and **fast** to perform, **highly sensitive**, actual test cost about $1.00 / for basic test, plus labor $0.50-$1.00; sample pooling can be used to reduce costs under some specific circumstances.

4) ELISA testing can be automated. This provides highly sensitive, relatively specific, and **high throughput screening.**

5) Low cross-reactivity: An ELISA test will detect one drug, plus possibly a few structurally related substances.

6) Highly cost-effective screening for **high potency drugs** (drugs administered at doses of less than 5.0 mg/horse).

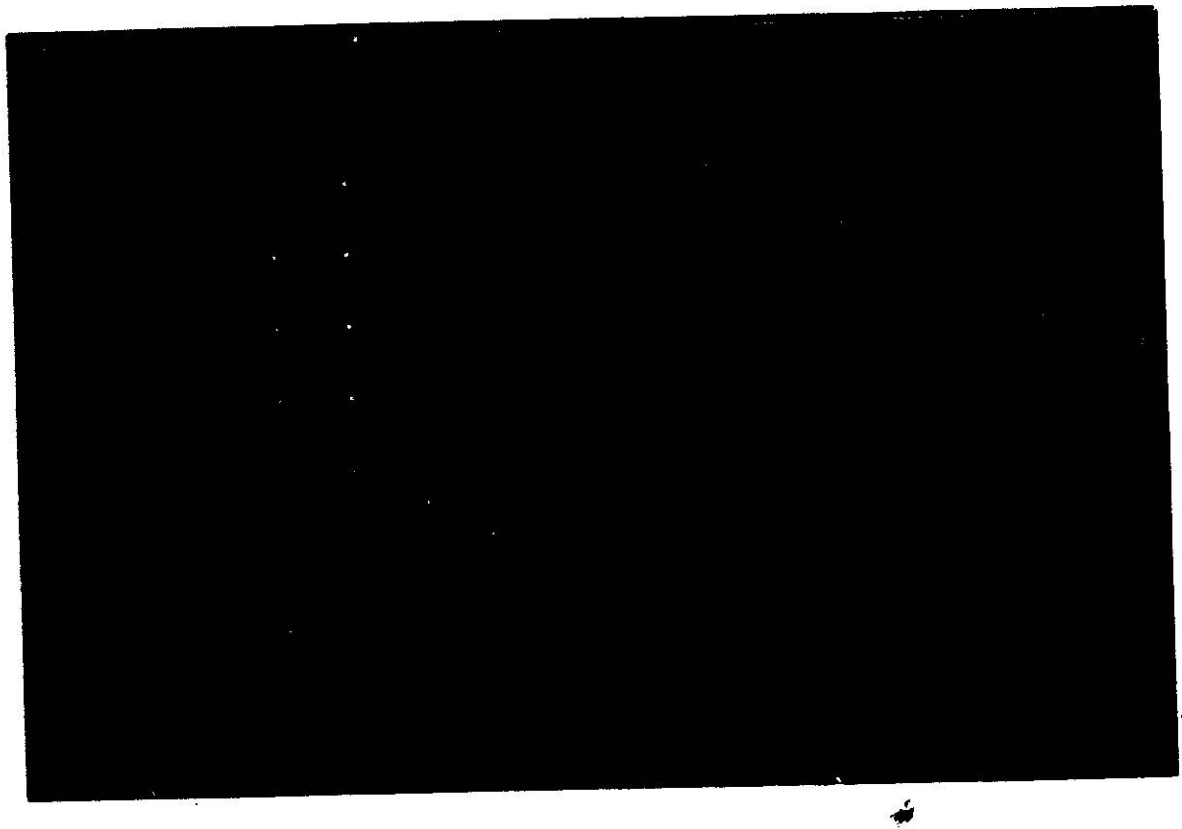
7) Many identifications of RCI Class 1, 2 & 3 drugs are based on ELISA screening.

8) ELISA tests to cover 100-150 drugs are currently available. More tests for certain analytes would be highly desirable.

9) Future goals for ELISA tests: More tests for specific drugs/analytes.

10) ELISA tests must be created individually on a drug by drug basis. A test costs $10-30K to develop. This process is specialized and generally outside of the skills and scope of analysts at the present time: ELISA tests are usually purchased as commercial kits.

11) Concern: The world budget for ELISA tests for use on racing samples is about $5M. New tests **do not increase total sales** for companies, they merely replace old tests: Therefore, a mechanism is needed to ensure that new test development continues.



A developed ELISA plate with reduced color or "positive" responses ("white-out" wells) showing on the left side of the plate in rows 1 and 2. Track samples, all "full blue" and "negative", are present on the right side of the plate from rows 3-11. As is apparent, ELISA plates can be "read by eye"; however an ELISA reader (cost, about $5-10K) provides a record for forensic purposes, quality assurance work, allows increased discrimination between positive and negative samples and also permits automation of the entire ELISA testing process.

THROUGHPUT

Limited primarily by cost. Between five and thirty ELISA tests can be run per sample for up to 100 to 600K individual tests / year.

ADVANTAGES:

1) Fast, resulting in high throughput.

2) High sensitivity and specificity.

3) Automatable,

4) Semi-quantitative.

5) Highly cost effective.

DISADVANTAGES:

1) Drug specific: thus a targeted analysis.

2) Limited number of useful assays available.

Industry Myth: Analysts have an ELISA for every known drug

INSTRUMENTAL ANALYSIS SCREENING:

Two "bench top" instruments suitable for use in instrumental screening: Above, a Gas Chromatograph-Mass Spectrometer (GC-MS), below a Liquid Chromatograph- Mass Spectrometer (LC-MS). Both instruments are equipped with an "autosampler" which means that they can be loaded with up to 50-100 samples that are analyzed over the next 8-12 hours (overnight) without further supervision (Figures courtesy of UC Davis, Equine Analytical Chemistry Laboratory).

STEP TWO (C): SCREENING BY INSTRUMENTAL ANALYSIS

1) This approach screens for drugs/analytes using **major laboratory instruments,** such as:

- High Performance Liquid Chromatography (HPLC)
- Gas Chromatograph- Mass Spectrometry (GC-MS)
- Liquid Chromatograph –Mass Spectrometry (LC-MS)

2) Hypothesis: Any drug should be detectable by these techniques with a relatively short test development time (<1 month).

3) Practice: Initial costs are higher but sample throughput can be high; since the processes are readily automated, the potential of these applications is very promising. However, at this time these are new procedures and their cost effectiveness is undetermined.

4) It should be noted that instrumental costs have declined steadily and that the ability to perform automated in-line analyses indicates high likelihood that this will be an increasingly important process for the future.

THROUGHPUT

Not yet determined. Assuming three instruments run in parallel, with an initial investment of ≥ $300,000 the number of potential drugs detected may run to 300 or more.

ADVANTAGES:

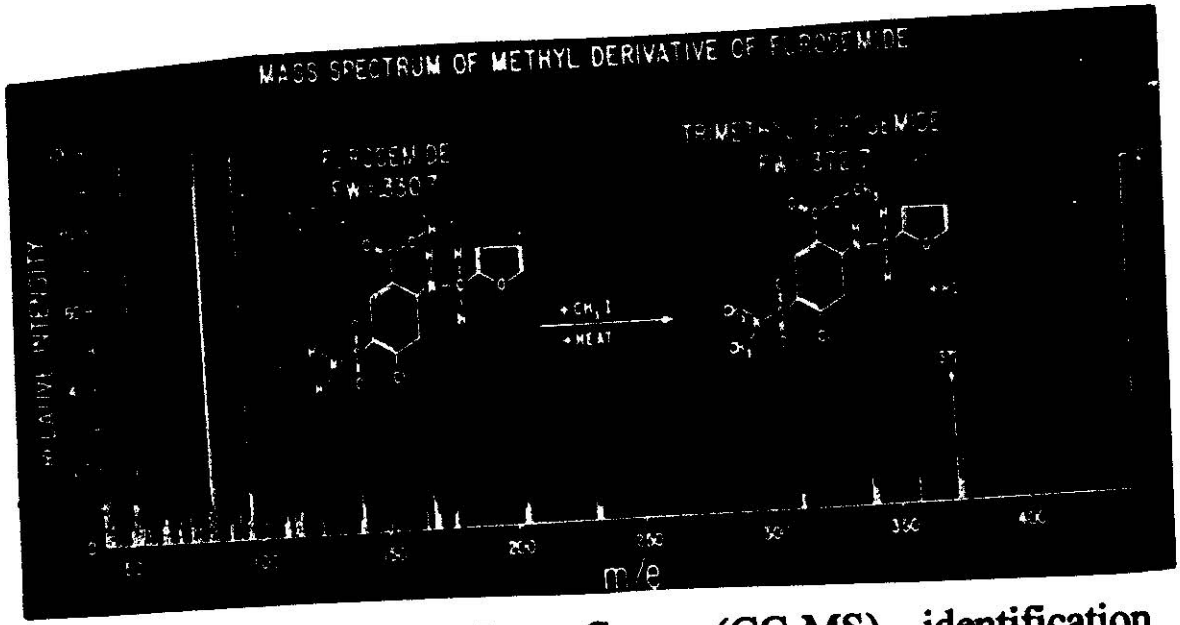
1) Potential to identify a wide range of drugs/analytes.
2) Suitable for automation.
3) High sensitivity and specificity.
4) Can be used for quantification
5) Mass Spectral methods may give molecular weight information which helps to identify "unknown" new drugs.

DISADVANTAGES:

1) Complex: Requires trained analysts to maintain and operate instruments.
2) These programs have high capital costs ($200-300K), high supply and instrument maintenance costs and high workforce costs: each instrument will cost at least $50K and at least three are needed. Each instrument may need an experienced analyst at another $40K/annum to run the instruments plus $15 K in supplies. Cost can come to $100K/year per instrument for total of $300K per year.

CONFIRMATION: MASS SPECTRAL (MS) ANAYSIS:
Liquid Chromatograph-Mass Spec-Mass Spec (LC-MS-MS)

Data System Operator Liquid Chromatograph MS-MS
Dr. Fritz Lehner operating the UK LC-MS-MS.



Gas Chromatography Mass Spec (GC-MS) identification of furosemide: Furosemide is **derivatized** with methyl iodide to form trimethyl furosemide. This step is necessary to enable gas chromatography and analysis. The reaction yields a molecular fingerprint of trimethyl furosemide.

STEP THREE: CONFIRMATION: MASS SPECTRAL (MS) ANAYSIS:

1) A full scan Mass Spectrum, properly obtained and independently confirmed is generally considered conclusive evidence that the identified drug/analyte is actually present in the sample. A mass spectrum is, in lay terms, equivalent to the **"fingerprint"** for identification of a drug/analyte. (See page 22)

2) A basic benchtop Gas Chromatograph-Mass Spectrometer (GC-MS) costs about $50-120K, has a 5-8 year life span and requires a full-time $40K analyst plus $10K in maintenance costs annually. This is a basic "work horse" confirmatory Mass Spectrometer. Laboratories need two or more of these instruments for modest workloads.

3) A full-scale Liquid Chromatograph-Mass Spec-Mass Spec (LC-MS-MS) is the state-of-the-art in its field for mass spectrometry. Because the input is through liquid chromatography, one can introduce a broader range of drugs/analytes than can be introduced into a traditional gas chromatograph-mass spectrometer, which requires that the drug be volatile in the carrier gas for analysis.

4) Cost of LC-MS-MS: $250K plus $50K mass spectrometry specialist, plus $30K per annum supplies/maintenance for optimal use of instrument. Estimated 5-8 year useful life.

5) Because a LC-MS-MS has the ability to separate and identify "parent" and "daughter" ions, the analyst can reduce background and potentially get higher sensitivity and specificity with LC-MS-MS.

THROUGHPUT

Low compared with the enormous screening throughput: Mass Spectral confirmation is the essence of forensic work. Samples for MS confirmation are very carefully selected and the work is very carefully performed with a view to successful regulatory action.

MASS FRAGMENTATION ("FINGERPRINT") OF DERIVATIZE ETORPHINE (ETORPHINE-TMS)

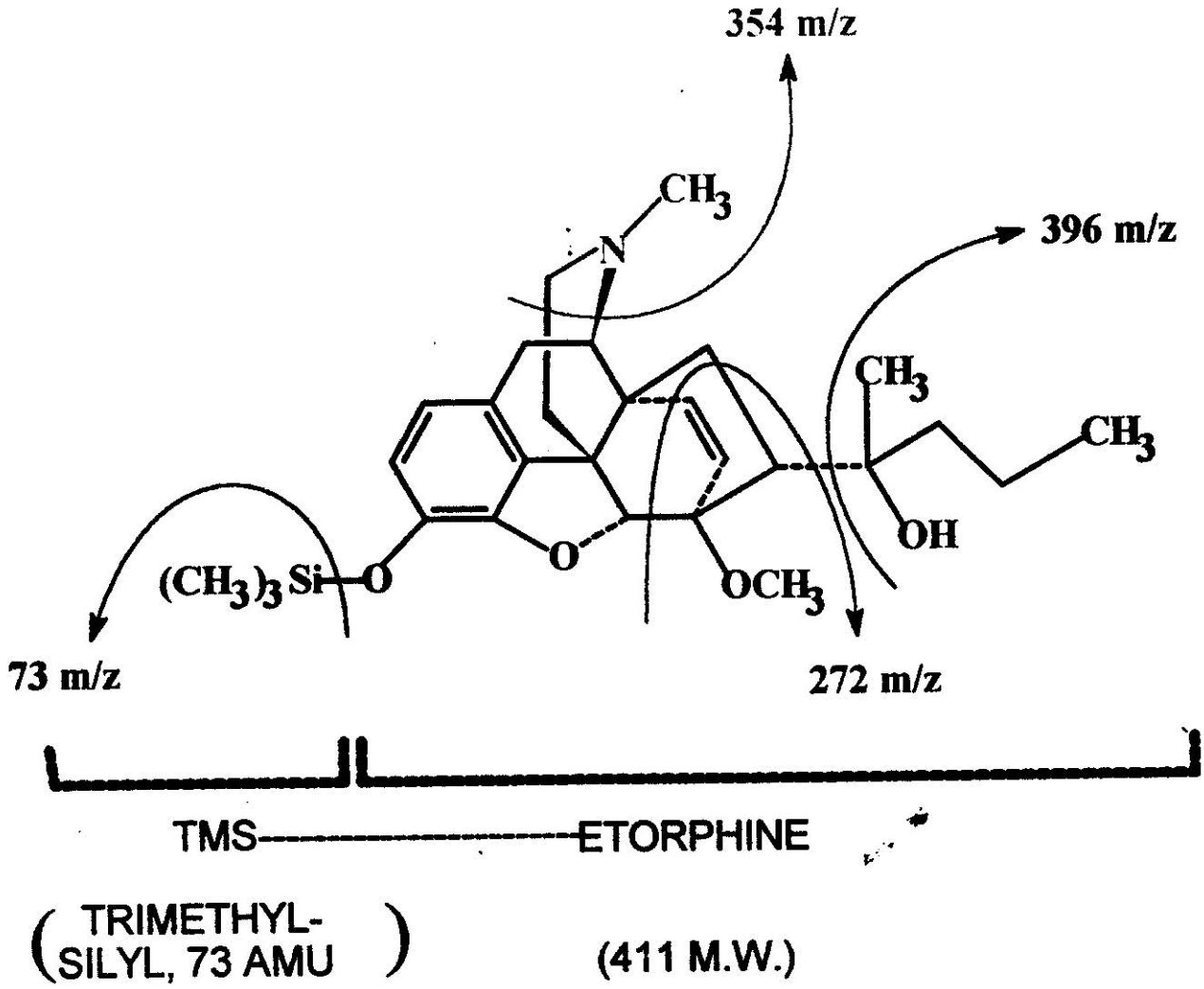


Table 1. Interpretation of principal peaks ("**fingerprint**") of etorphine-TMS Mass Spectrum

Peak (m/z)	Interpretation
483	Etorphine-TMs
468	(483) minus methyl group (-15)
396	(483) minus isopentanol group (-87)
354	(396) minus $HC{=}NCH_3$ group (-42)
323	(396) minus TMS group (-73)
272	(354) minus methyoxethenylmethylene group (-82)
73	TMS group (483 minus 410)

MASS SPECTRAL CONFIRMATION OF ETORPHINE, "ELEPHANT JUICE" IN A POST-RACE SAMPLE

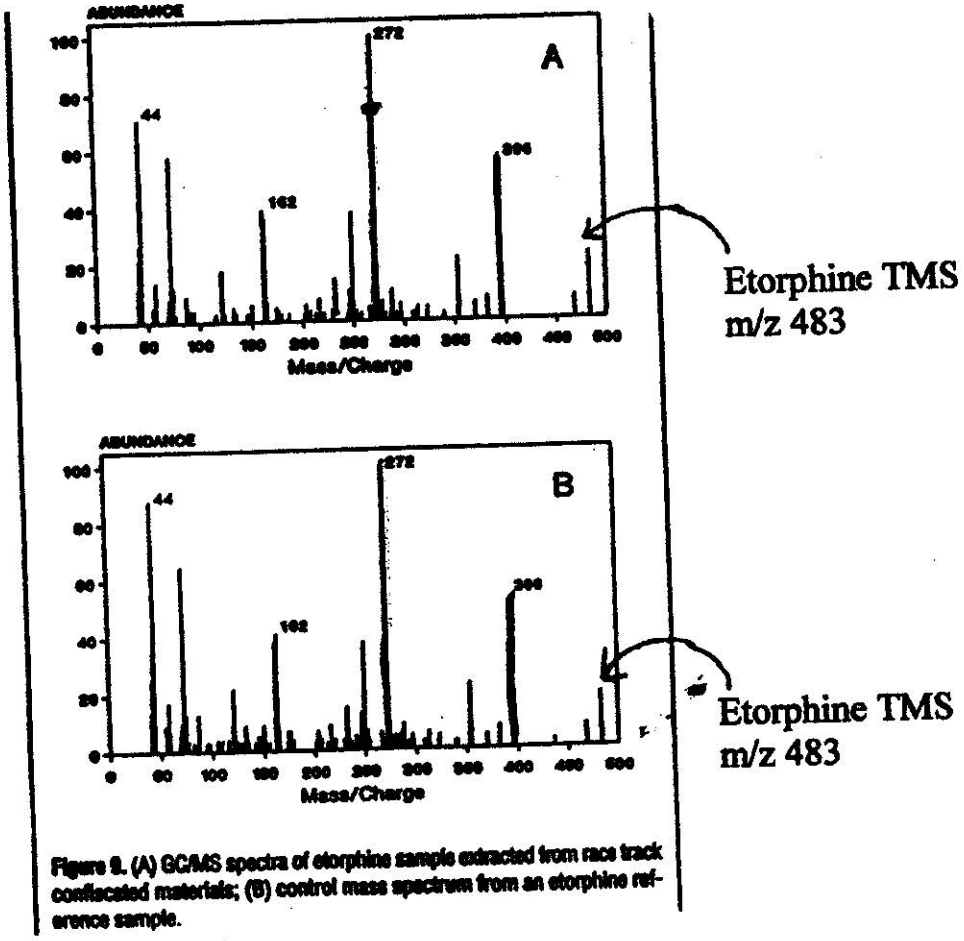


Figure 9. (A) GC/MS spectra of etorphine sample extracted from race track confiscated materials; (B) control mass spectrum from an etorphine reference sample.

Comparison of Mass Spectra of track confiscated etorphine and an authentic standard. The lower figure shows the mass spectrum of an **authentic derivatized etorphine laboratory standard.** Note the "molecular ion" at about a mass 483, the "base peak" at mass 272 and the various other ions of this standard or control spectrum. Note the very close correspondence of this "standard" mass spectrum with the mass spectrum of the material recovered from the track sample, indicating that the material recovered is indistinguishable from authentic etorphine.

DRUG "POTENCY" AND DRUG TESTING

1) High Potency Drugs:
Dose = 50 micrograms (Etorphine) — Clenbuterol/Etorphine
TLC not effective

250 micrograms (Clenbuterol) — TLC rarely effective

2) Intermediate Potency Drugs:
Dose = 250 mg:
(250,000 micrograms) — Furosemide
Easily detected

3) Low Potency Drugs:
Dose = 2.5 Grams/Horse:
(2,500,000 micrograms) — Phenylbutazone
Very easily detected

POTENCY: Since the dose of etorphine is 1/50,000 of the dose of phenylbutazone, etorphine is 50,000 times more potent than phenylbutazone. Etorphine is therefore considerably more difficult to detect than phenylbutazone.

"RULE OF THUMB": If the dose/horse is less than 5 mg/horse, then the drug is a high potency agent that will likely be difficult to detect by Thin Layer Chromatography.

URINE TESTING AND HIGH POTENCY DRUGS: Because the doses (quantities) of high potency drugs administered are so small, the concentration of these drugs / drug metabolites by the kidney is very important for detection of these agents in the urine.

STEP FOUR: INDEPENDENT CONFIRMATION: "SPLIT" OR "REFEREE"SAMPLE ANAYSIS

Where the governing regulations permit, independent confirmation of the Mass Spectral findings can be developed by submitting a **"split"** or **"referee"** sample to a referee laboratory. The horseman is generally provided with a list of Commission approved laboratories from which to choose the **"referee"** laboratory. Independent confirmation verifies the chemical evidence and substantially reduces the likelihood that the analytical data will be challenged. The matter then generally proceeds to regulatory review.

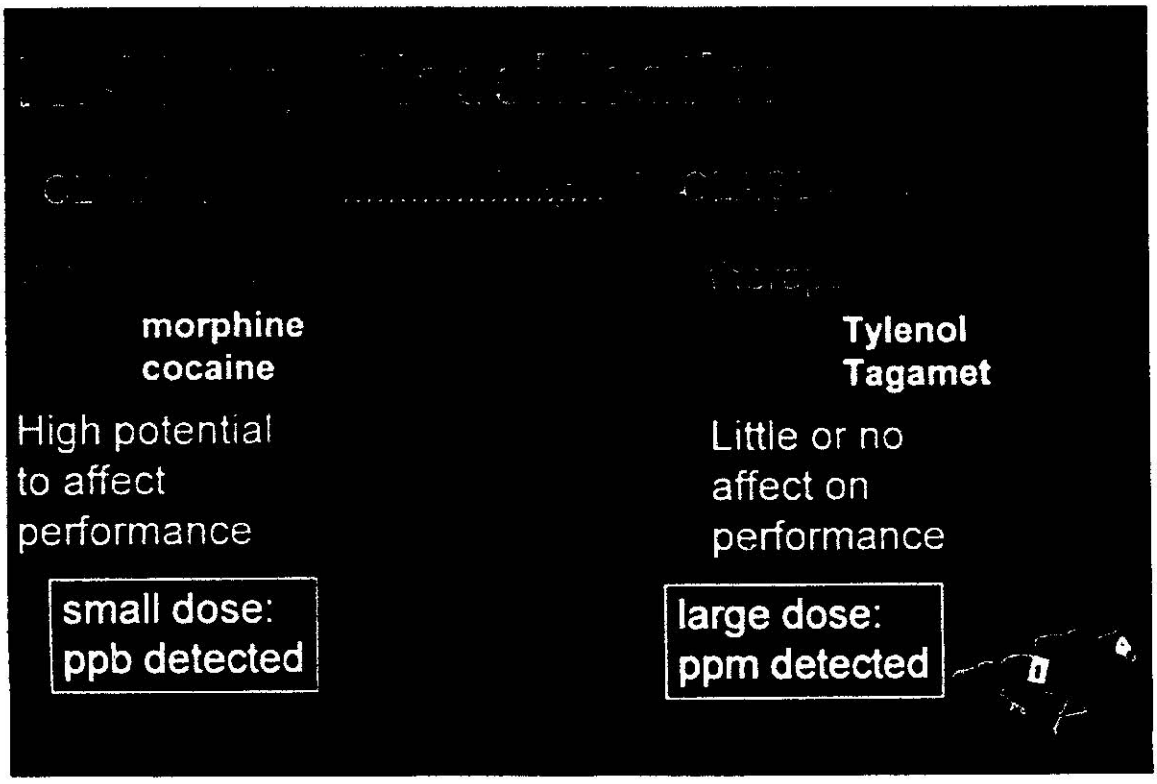
STEP FIVE: REGULATORY REVIEW:

Factors involved in regulatory review include the **Association of Racing Commissioners International (ARCI) classification** of the drug. Under this system performance altering drugs are divided into five classes. Class one drugs have the **highest ability to affect performance** and carry the highest penalties. Numerically higher class drugs (classes 2, 3, 4, and 5) have lesser abilities to affect performance and carry lower penalties. Other factors include the possibility that the drug or analyte detected represents a sub-therapeutic residue of the therapeutic medication or other mitigating factors.

Association of Racing Commissioners International, Inc.
Drug Testing Standards and Practices Program
Model Rules Guidelines

Uniform Classification Guidelines for Foreign Substances and Recommended Penalties and Model Rule

Revised October 1996



APPENDIX I

ARCI FOREIGN SUBSTANCES CLASSIFICATION SYSTEM

PURPOSE: (652 drugs)	Classifies 600 plus drugs into 5 classes: simplifies dialog, both scientific and lay, about these agents.
Class 1 (38 drugs)	Stimulants and depressants: Highest potential to affect performance: No therapeutic uses in racing horses.
Examples:	Opiates, Cocaine, Amphetamines.
Class 2 (296 drugs)	High potential to affect performance: Few or no generally accepted therapeutic uses in racing horses.
Examples:	Local anesthetics, barbiturates, diazepam.
Class 3 (122 drugs)	Pharmacology suggests lesser ability to affect performance; may or may not have generally accepted medical uses in racing horses.
Examples:	Tranquilizers, anti-histamines, bronchodilators, procaine.
Class 4 (176 drugs)	Therapeutic drugs with less potential to affect performance than Class 3.
Examples:	Non-steroidal anti-inflammatories, corticosteroids, isoxsuprine, methocarbamol.
Class 5 (20 drugs)	Therapeutic drugs with less potential to affect performance than in class 4, masking substances and miscellaneous agents.
Examples:	DMSO, cimetidine, cromolyn, polyethylene glycol, warfarin

ARCI/Classification Committee: Drs. Short, Sams, Soma and Tobin

SIGNIFICANCE: A highly successful system that enables rational comparisons and discussion of these drugs by administrators, scientists and regulators.

EQUINE DRUG AND METABOLITE STANDARDS

by

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No	METABOLITE / DEUTERATED STANDARD	PARENT DRUG	REMARKS
1.	3-HYDROXYLIDOCAINE	LIDOCAINE	Metabolite
2.	4-HYDROXYLIDOCAINE	LIDOCAINE	Metabolite
3.	3-HYDROXYMEPIVACAINE	MEPIVACAINE	Metabolite
4.	4-HYDROXYMEPIVACAINE	MEPIVACAINE	Metabolite
5.	3-HYDROXYBUPIVACAINE	BUPIVACAINE	Metabolite
6.	3-HYDROXYROPIVACAINE	ROPIVACAINE	Metabolite
7.	4-HYDROXYROPIVACAINE	ROPIVACAINE	Metabolite
8.	O-DESMETHYLPYRILAMINE	PYRILAMINE	Metabolite
9.	2-(1-HYDROXYETHYL)PROMAZINE SULFOXIDE	ACEPROMAZINE	Metabolite
10.	(1-HYDROXYETHYL)PROMAZINE (uncrystallized)	ACEPROMAZINE	Metabolite
11.	PROMETHAZINE SULFOXIDE	PROMETHAZINE	Metabolite
12.	3-HYDROXYPROMAZINE	PROMAZINE	Metabolite
13.	2-(1-HYDROXYPROPYL)PROMAZINE SULFOXIDE	PROPIONYLPROMAZINE	Metabolite
14.	4-HYDROXYPROPANOLOL	PROPRANOLOL	Metabolite
15.	2-(2-AMINOETHYL)-3-(4-CHLOROPHE-NYL)-3-HYDROXY-2,3-DIHYDRO-ISO-INDOL-1-ONE	MAZINDOL	Metabolite
16.	3-HYDROXYTRIPELENNAMINE	TRIPELENNAMINE	drug derivative
17.	7-HYDROXYCHLORPROMAZINE	CHLORPROMAZINE	Metabolite
18.	PHENYLBUTAZONE-D_9	PHENYLBUTAZONE	Deuterated standard
19.	PROCAINE-D_{10}	PROCAINE	Deuterated standard
20.	FUROSEMIDE-D_5	FUROSEMIDE	Deuterated standard

STANDARDIZED TESTING:

1) **Standardized testing** is only needed for **subtherapeutic residues of legitimate therapeutic medications, dietary substances and environmental contaminants** likely to be detected in post-race urine samples. For other drugs/analytes there is no need for limits on testing sensitivity.

2) There are various approaches to **"standardized testing,"** in place world-wide, as follows:

3) Specified **"limits", "cut-offs"** or concentration or **"Thresholds"** in plasma or urine. About 10 thresholds for dietary substances and environmental substances are well established internationally. Additionally, phenylbutazone & Lasix® thresholds are well established in many U.S. jurisdictions.

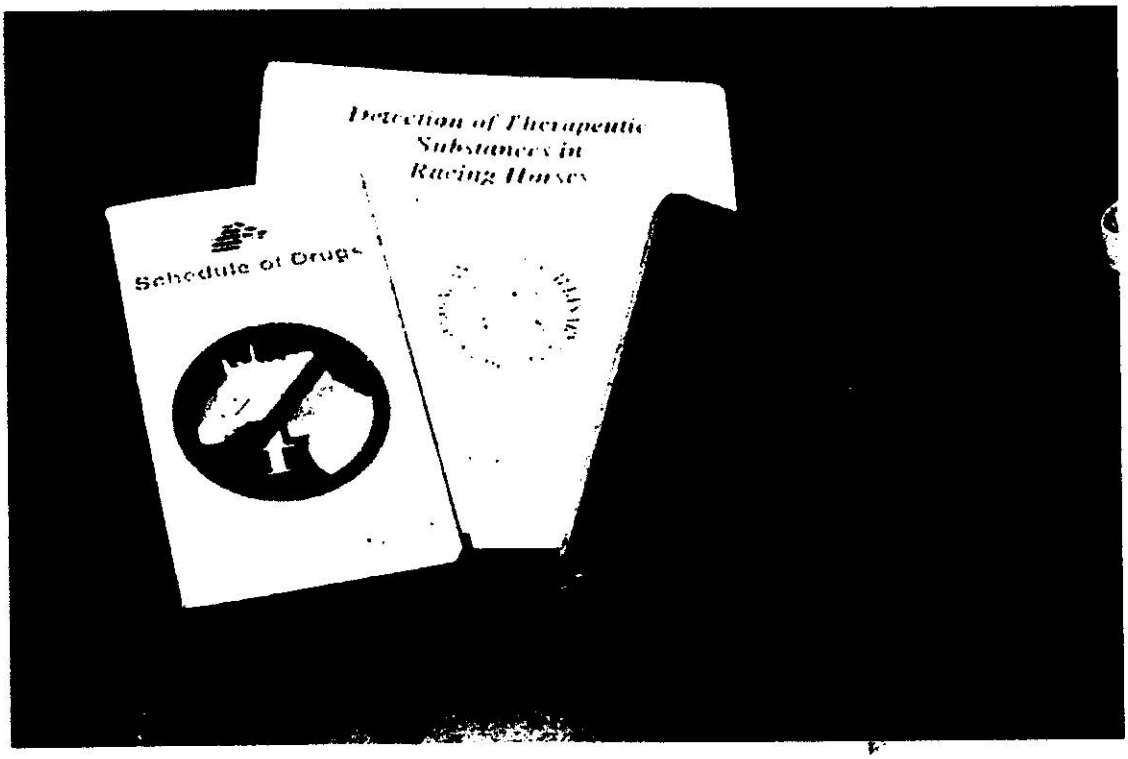
4) **"Decision levels",** effectively eight urinary **"limits" or "cut-offs"** are regulated by the California Horse Racing Board.

5) **"Detection Times"** Canada, Australia, and, most recently, Western Europe have established "Detection Times" booklets for about 70 therapeutic medications. The Canadians have linked these detection times to loss of therapeutic effect, apparently based on clinical experience.*

6) Why so many similar approaches with **different answers** world-wide? Answer: Some drugs are found in urine as unique drug metabolites: chemical synthesis of these metabolites is generally outside the professional skills of analysts, so appropriate **chemical standards** for some analytes have not been available.

7) Partial solution: synthesis of these **equine drug metabolites / standards,** as attached, has the potential to standardize testing for therapeutic medications worldwide.

*Dr. Mike Weber, Agriculture Canada, Personal Communication, 1998.



Published **"Detection Times"** booklets from Agriculture Canada, The Australian Equine Veterinary Association, and the European Horserace Scientific Liaison Committee (EHSLC). Because "detection times" vary with the Limit of Detection (LOD), (sensitivity) of the analytical methods, **analytical methods must be "frozen"** or standardized at a given sensitivity for "detection times" to be valid. Such standardization is acknowledged by the **Canadian** and **Australian** authorities.

APPENDIX III

CURRENT RESEARCH PROBLEMS IN EQUINE FORENSIC SCIENCE

1) **NEW BIOTECHNOLOGY PRODUCTS:** It appears that entirely new techniques and approaches are needed for biotechnology products such as equine growth hormone and erythropoietin. Some of these problems may be solved by LC-MS-MS.

2) **OLD "SMALL MOLECULE" DRUGS:** Vigorously pursue current approaches to generally available drugs and medications: More ELISA tests and improved **"high throughput"** screening procedures, such as instrumental screening, which approaches have been highly productive in the past.

3) **STANDARDIZED TESTING:** For legitimate therapeutic medications: Clear guidelines for veterinarians to identify and prevent the unproductive reporting of "sub-therapeutic residues" of therapeutic medications.

4) **AN "IN PLACE" PROTOTYPE:** The Canadian approach to standardized testing, which has worked successfully in Canada. Helpful in developing and improving this type of approach would be a centralized authority and better scientific approaches to defining a, "sub-therapeutic residue".

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