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QUANTIFICATION AND CONFIRMATION OF FLUNIXIN IN EQUINE PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ION TRAP MASS SPECTROMETRY

DEVELOPED BY

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INTRODUCTION

Flunixin (Banamine®) is a non-steroidal anti-inflammatory agent with a tolerance level of 10 ng/mL in plasma on race day in Pennsylvania, based on strong scientific data⁽¹²⁾, established by the Pennsylvania Racing Commission. Previous methods for the quantification of Flunixin in plasma include High Performance Liquid Chromatography^(7,9-13) (HPLC), Thin-Layer Chromatographic Densitometry (TLC)^(6,10), and Gas Chromatography-Mass Spectrometry (GC/MS)^(4,5,6,8). TLC and HPLC required subsequent confirmation by mass spectrometry; while, GC/MS was not sufficiently accurate and precise for quantitative determinations at this threshold. The use of High Performance Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) allows the determination of Flunixin to an LOQ an order of magnitude lower than the tolerance level of 10 ng/mL, thus increasing the confidence of the determinations for race day plasma concentrations of Flunixin. It is for this reason that this Standard Operating Procedure was developed for the qualitative and quantitative determination of Flunixin in equine plasma.

SCOPE

This standard operating procedure describes the quantification and confirmation of Flunixin in equine plasma previously screened by Enzyme-Linked Immunosorbent Assay. Liquid-liquid extraction of equine plasma is employed with subsequent analysis on a Thermo Electron Deca XP Plus ion trap mass spectrometer. This method can be directly used as an instrumental screening method by appropriate alteration of the sample injection sequence. Any concentration of Flunixin in equine plasma that does not meet the criteria presented by this SOP for reporting such a positive finding to the Racing Commissions will be considered a negative finding and will be so reported to the appropriate Racing Commission.

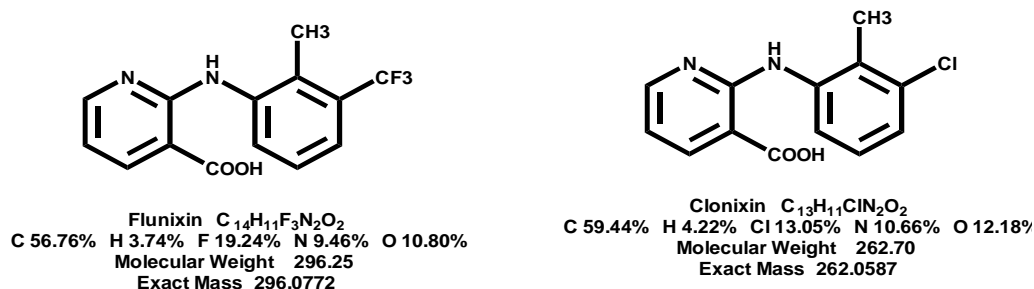


Figure 1. Structures of Flunixin and Clonixin (Internal Standard)

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PRINCIPLE OF METHOD

The plasma sample is diluted with phosphate buffer (pH 3.0) and subjected to liquid-liquid extraction using methyl tert-butyl ether. The residue is reconstituted in 0.1% aqueous formic acid and analyzed by positive ion electrospray LC/MS/MS. The concentration of Flunixin is determined by the peak area ratio of the extracted product ions from the full scan MS/MS spectra of Flunixin and Clonixin.

PRIMARY REFERENCE STOCK SOLUTIONS

Primary Analytical Standard Reference Material

Flunixin Meglumine salt, (**USP, Cat. # 27460**).
Formula Weight: 491.47, Flunixin Free Acid Formula Weight: 296.08
Salt Equivalent of Free MW/Salt MW=1.66

Primary Analytical Internal Standard Reference Material

Clonixin, (**Schering Research Division, Cat. #. Sch 10304**).
Formula Weight: 262.70

Obtain these materials from the QAO. Record accession of these materials on the pharmacy log sheet.

I. PREPARATION OF PRIMARY REFERENCE STOCK SOLUTIONS

Flunixin

Weigh between 5 and 10 mg (X.xx mg) Flunixin salt into a glass bottle.
Dilute to volume using HPLC grade (or better) methanol (Volume **Y.yy** mL = **X.xx/1.66**).
Cap and mix until Flunixin salt is completely dissolved in methanol.

The resulting concentration of Flunixin is 1 mg/mL.
Store at approximately 4 °C.

***Complete Balance Use Log and QA Primary Reference Standard Log for this process.
Label the primary reference stock solutions with QA Primary Reference Log SR# (i.e. SR# 637 and Primary Reference Powder Designation (i.e. R-Flun-3).***

Clonixin

Weigh out between 5 and 10 mg (X.xx mg) Clonixin into a glass bottle.
Dilute to volume using HPLC grade (or better) methanol (Volume **Y.yy** mL = **X.xx**).
Cap and mix until Clonixin is completely dissolved in methanol.

The resulting concentration of Clonixin is 1 mg/mL
Store at approximately 4 °C (refrigerator).

***Complete Balance Use Log and QA Primary Reference Standard Log for this process.
Label the primary reference stock solutions with QA Primary Reference Log SR# (i.e. SR# 638) and Primary Reference Powder Designation (i.e. R-Clon-1).***

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Using the following reference powder:

Drug	Source	Cat #	Lot #	QA Reference Label
Flunixin Meglumine	USP	27460	F	R-FLUX-2
Clonixin	Schering	Sch-10304		R-CLONX-1

1mg/mL MeOH Stock Solutions were prepared as follows:

Drug	Using	mg Used	mL MeOH	Label
Flunixin	R-FLUX-2	7.7	4.64	Flun012704Stock
Clonixin	R-CLONX-1	6.9	6.9	Clon012704Stock

II. PREPARATION OF SECONDARY REFERENCE STOCK SOLUTIONS

Materials Needed: Flunixin Primary Reference Stock (1 mg/mL)
 Acetonitrile: Water: Formic Acid (50:50:1).
 0.1% Formic Acid (aqueous)

Prepare Flunixin Secondary Reference Stock solutions according to Table 1:
 From these, the following 10 ug/mL FIA solutions were prepared in 50:50:1:

Drug	Using	uL Used	mL 50:50:1	Label
Flunixin	Flun012704Stock	100	9.9	FlunFIA012704
Clonixin	Clon012704Stock	100	9.9	ClonFIA012704

From these, the following 100 ng/mL 0.1% Formic Acid LC solutions were prepared:

Drug	Using	uL Used	mL 0.1% FA	Label
Flunixin	FlunFIA012704	100	9.9	FlunLC012704
Clonixin	ClonFIA012705	100	9.9	ClonLC012704

Label secondary reference stock solutions with the Label designated in Table 1 and record preparation and labeling in the secondary preparation logbook in the appropriate Unit of the Laboratory.

III. PREPARATION OF CALIBRATOR SECONDARY STOCK SOLUTIONS

The following calibrators are prepared in HPLC grade (or better) water.

Calibrator Code #	Used For: (ng/mL)	uL	Using:	mL HPLC Water
031204FLN1000	100	1000	FlunFIA012704	9
031204FLN750	75	750	FlunFIA012704	9.25
031204FLN500	50	500	FlunFIA012704	9.5
031204FLN200	20	200	FlunFIA012704	9.8
031204FLN100	10	1000	031204FLN1000	9
031204FLN50	5	1000	031204FLN500	9
031204FLN10	1	1000	031204FLN100	9

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Record the preparation and labeling in the secondary preparation logbook in the appropriate Unit of the Laboratory.

Prepare 16x125 mm screw cap culture from Table 3 for preparation of secondary stock calibrators. Print labels for each calibrator of Table 3.

**LABEL (format MMDDYYFLNNG/ML) and (format MMDDYYFLNNG/MLQC)
Print using AVERY Template 5267 in MS Word) 14 of each**

Label screw cap culture tubes (16x125 mm) in 14 x 14 format, and aliquot 1 mL of the appropriate calibrator from Table 3 into each respective tube. Cap and store at 4° C.

IV. PREPARATION OF CLONIXIN INTERNAL STANDARD WORKING SOLUTION

Materials needed: 1 mg/mL of Clonixin primary reference stock
1 ug/mL Internal standard solution, using 10 ug/mL Clonixin (ClonFIA012705)

Drug	Using	uL Used	ml HPLC Water	Label
Clonixin	ClonFIA012705	1000	9	ClonIS012704

Procedure:

Dilute 100 uL of 10 µg/mL Clonixin standard stock solution in a glass vial to volume using 9.9 mL of Water. Mix.

The final concentration of Clonixin is 25 µg/mL.

Storage Requirements

Store at approximately 4 °C (refrigerator).

V. SAMPLE REQUIREMENTS FOR ANALYSIS

Prepare Calibrators, Quality Control samples (including negative control), and suspect samples (in triplicate) for each analysis performed.

VI. SAMPLE EXTRACTION BY LIQUID-LIQUID EXTRACTION

1. Dispense 1 mL suspect sample (in triplicate) into individual clean, labeled 16x125 mm screw cap culture tubes and 1 mL negative control plasma into appropriately labeled tubes for negative controls, positive controls, and calibrators.
2. Add 2.0 mL of saturated pH 3.0 phosphate buffer into each tube, vortex.
3. Add 100 µL of the secondary calibrator stocks to the appropriately labeled calibrator and control tubes.
4. Add 100 µL of internal standard working solution to all tubes except NC1
5. Add 5 mL of methyl tert-butyl ether (MTBE) into each tube (calibrators, controls, and samples) and extract for 10 minutes by mixing on a rotorack. Centrifuge at 3000 rpm for 10 minutes.
6. Transfer each top MTBE layer into a clean, labeled culture tube.
7. Evaporate to dryness at 55-75 °C under nitrogen or air.
8. Reconstitute the residues with 100 uL of 0.1% Formic Acid (aqueous)

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9. Transfer the above solution into labeled auto sampler vials fitted with limited volume inserts, then cap. All the samples are ready for LC/MS/MS analysis.

VII INSTRUMENT CONDITIONS

Mass Spectrometer: Thermo Electron Deca XP Plus Ion Trap

Deca XP Plus Scan Event Settings

Scan Event Details:

- 1: + c norm ·(297.1)->oW(180.0-300.0)
MS/MS: Amp. 45.0% Q 0.250 Time 30.000 IsoW 1.3
- 2: + c norm ·(263.1)->oW(180.0-300.0)
MS/MS: Amp. 45.0% Q 0.250 Time 30.000 IsoW 1.3

Liquid Chromatograph: Thermo Electron Surveyor

Solvent A: 2.33 mM Formic Acid pH 5
Solvent B: Acetonitrile 0.1% Formic Acid

Column: ACE 5u C-18 50 x 3 mm (MacMod ACE-121-0503)

Injection Mode: Partial Loop using a 20 µL Sample Loop

Injection Volume: 10 µL.

System Flush and Wash: 2.3 mM Formic Acid pH 5

Solvent Blank: 0.1% Formic Acid (aqueous)

Gradient Table

	Time, min	% A	% B	Flow, uL/min
1	0	80	20	20
2	0.2	80	20	20
3	0.3	80	20	200
4	0.5	80	20	200
5	1	0	100	200
6	4.5	0	100	200
7	4.51	80	20	400
8	5.9	80	20	400
9	5.91	80	20	20
10	6.2	80	20	20

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VIII. SAMPLE LIST SETUP FOR FLUNIXIN ANALYSIS

A. The sequence order for screening and confirmation is the same, except for unknown samples. Screening samples are sequentially ordered by track and sample, with blanks bracketing the individual track sets. Confirmation is independent, repeat preparation of all QC and calibrators, with target samples prepared in triplicate, and triplicates bracketed by blanks.

1. Blank
2. Column Test
3. Blank
4. Negative Control
5. Negative Control +Internal Standard
6. QC1
7. QC2
8. QC3 (optional)
9. Blank
10. C1
11. C2
12. C3

13. C4
14. C5
15. C6
16. C7

17. Blank 18. Track A, Samples 1 thru N 19. Blank

20. Repeat 17 thru 19 as needed

21. Blank
22. QC1
23. QC2
24. QC3 (optional)
25. Blank
26. C1
27. C2
28. C3
29. C4
30. C5
31. C6
32. C7
33. Blank

Blank Track A, Sample X ₁ Track A, Sample X ₂ Track A, Sample X ₃ Blank
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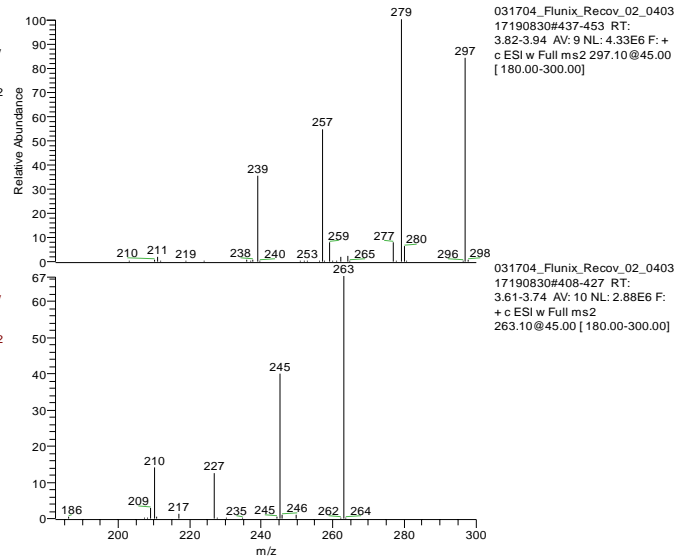
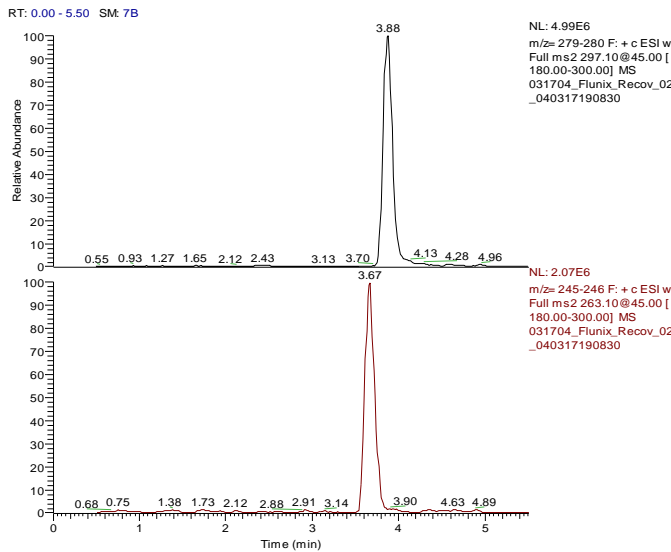
Repeat as needed for the number of samples for confirmation

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34. Blank-Standby Method

Screening analysis uses no waste injection, to allow for repeat analysis in case of power failure, sequence error, retention drift, or other unforeseen need for reanalysis. Confirmation uses partial loop injection due to lower sample throughput and better repeatability statistics at these concentrations.

IX. CHROMATOGRAMS AND SPECTRA

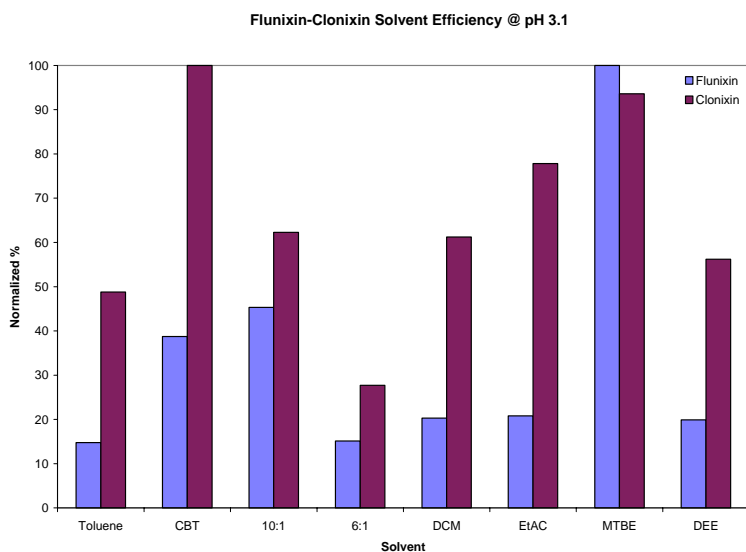


Chromatograms for Flunixin (top) and Clonixin (bottom)

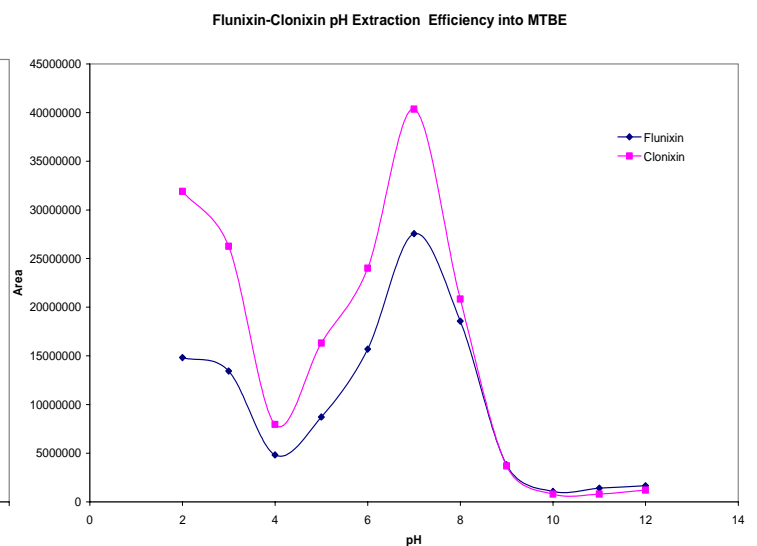
MSMS spectra for Flunixin (top) and Clonixin (bottom)

X. METHOD VALIDATION

Extraction Conditions



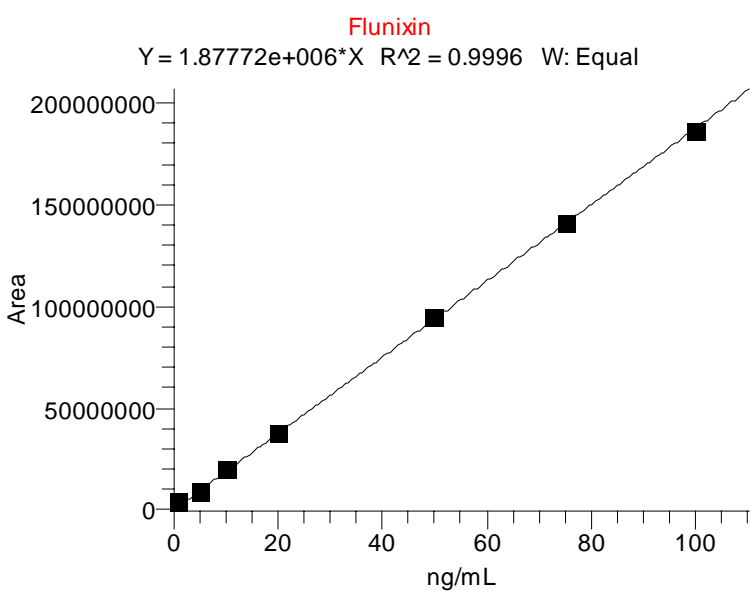
Solvent Extraction Efficiency for Flunixin and Clonixin at pH 3.1



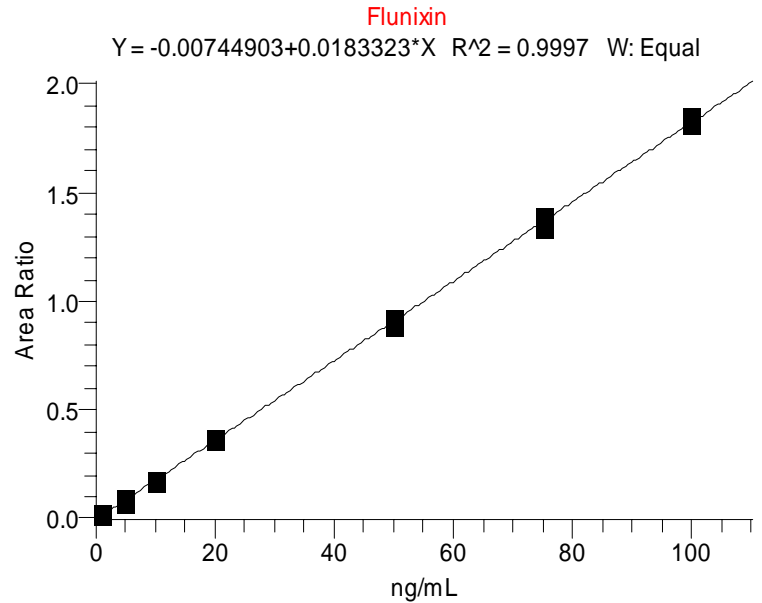
pH Extraction Efficiency for Flunixin and Clonixin using methyl tert-butyl

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Calibrator Preparation

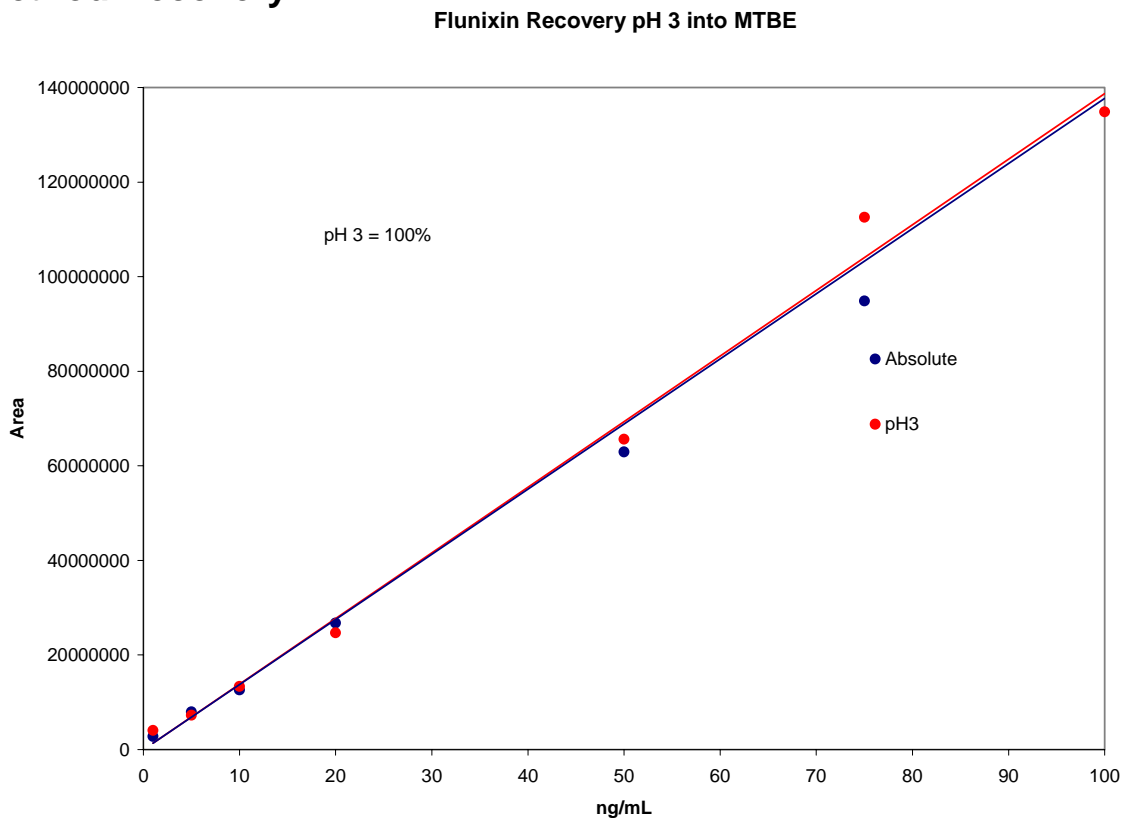


Calibrator Solutions Linearity, analyzed as neat unextracted solutions



Extracted plasma spiked calibrator series Linearity, n=4

Method Recovery

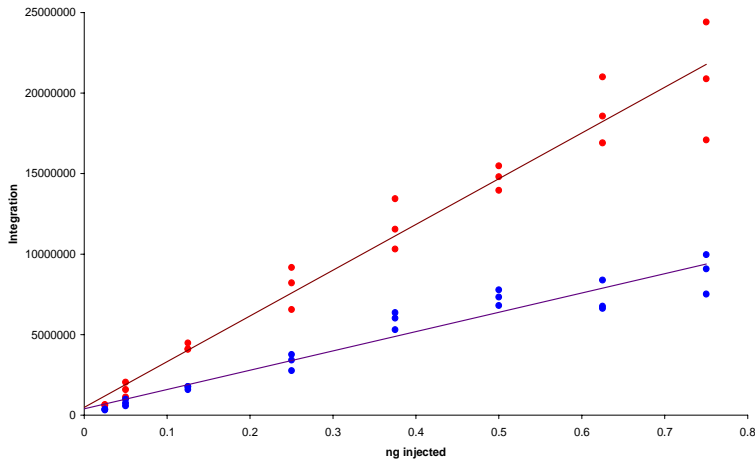


Plasma extraction efficiency at pH 3.0 into MTBE versus unextracted Flunixin

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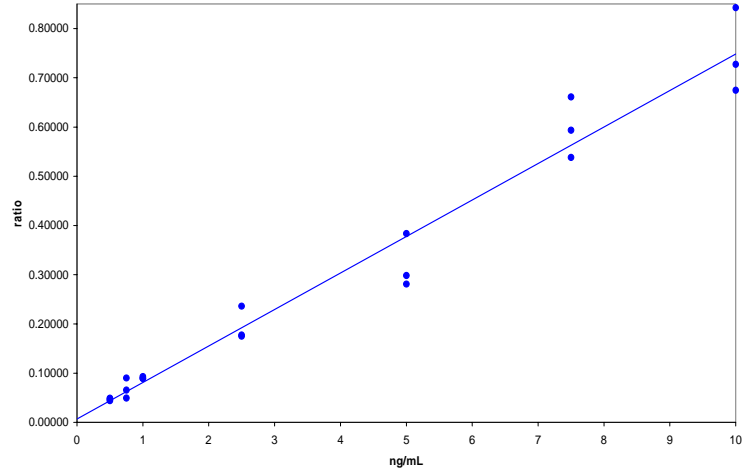
Limit of Detection (Instrument and Method)

Flunixin-Clonixin Variable Volume Partial Loop Injection
Instrument Limit of Detection (LOD_i)



LOD_i for Flunixin and Clonixin ~ 50 pg injected

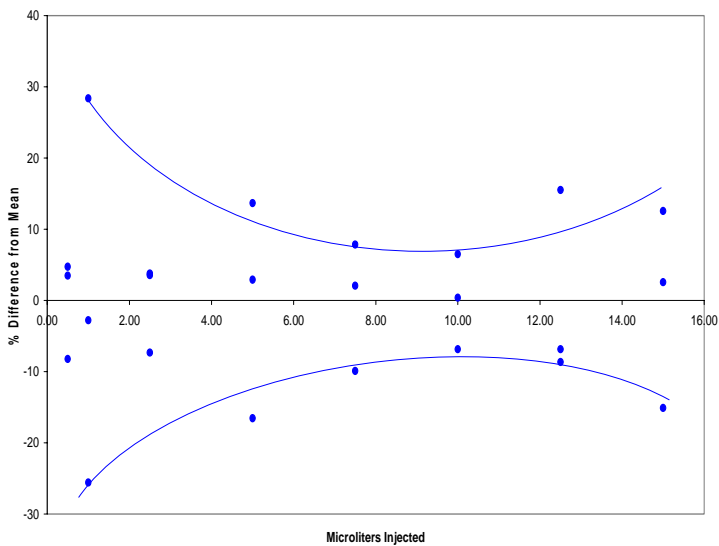
Flunixin Limit of Detection - Method LOD_m 0.5 ng/mL
Deca XP Plus 10uL of 100 uL of 0.5 ng/mL = ~ 50 pg injected



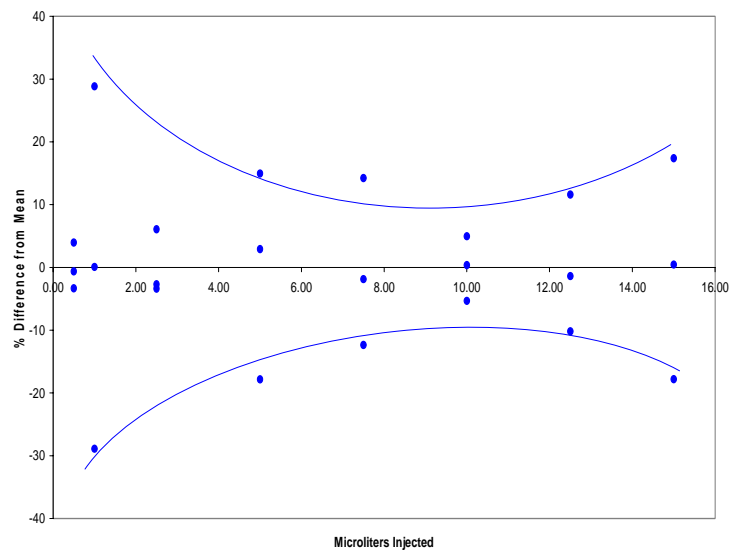
LOD_m for Flunixin ~ 50 pg injected (10 uL of 100 uL from 500 pg/mL)

Optimum Repeatability of Injection Volumes

Clonixin Partial Loop Injection - Variable Volume Confidence
Best Injection Volume ~ 10 uL



Flunixin Partial Loop Injection - Variable Volume Confidence
Best Injection Volume ~ 10 uL



Normalized Difference from Mean plots for variable volume injections of Clonixin (left) and Flunixin (right). Optimum injection volume in partial loop mode is 10 uL.

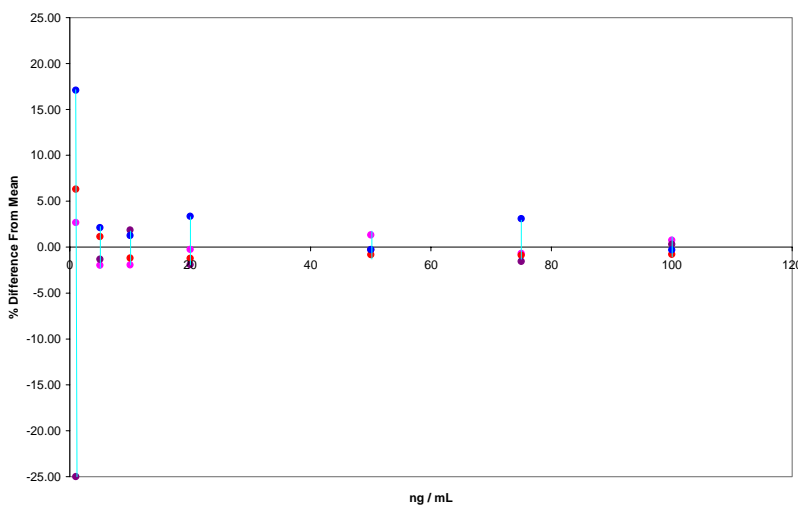
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Method Precision and Accuracy

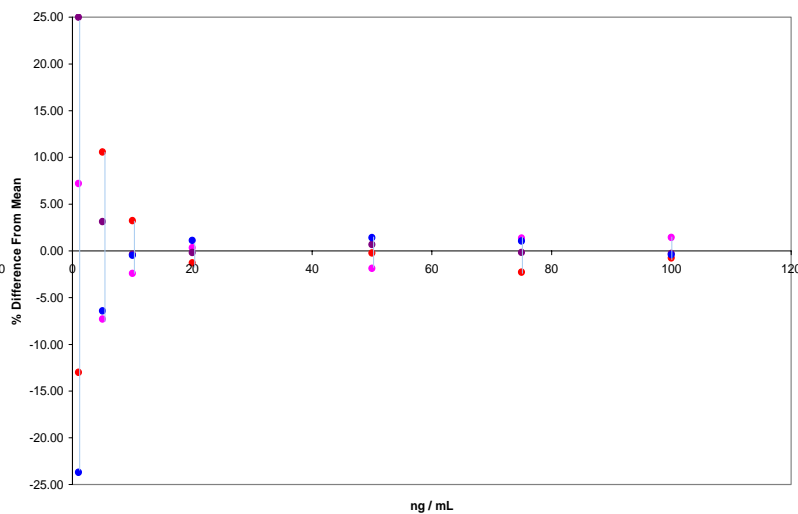
ng/mL	Within Run CV	Within Run Accuracy %	Between Run CV	Between Run Accuracy %
1	18.44	177.60	24.52	128.00
5	1.96	101.47	8.49	96.11
10	1.85	101.65	2.35	98.15
20	2.34	99.52	1.01	100.14
50	0.93	100.05	1.42	99.72
75	2.10	101.35	1.65	100.11
100	0.67	100.54	0.98	100.03

Inter-assay and Intra-assay precision and accuracy across the calibration range (left)

Inter Assay Flunixin Precision Deca XP n=4

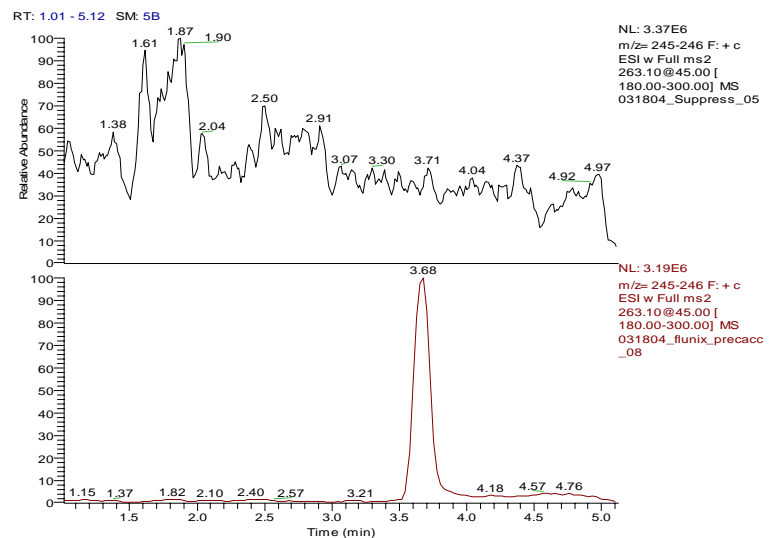
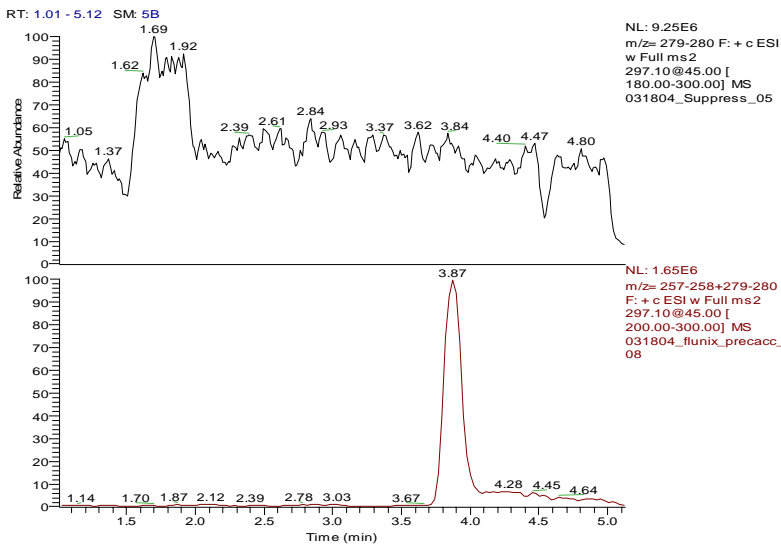


Intra Assay Flunixin Precision Deca XP n=4



Inter-assay (left) and Intra-assay (right) precision across the calibration range (above). These data indicate a lower limit of quantitation (LLOQ) of ~ 1 ng/mL (100 pg injected).

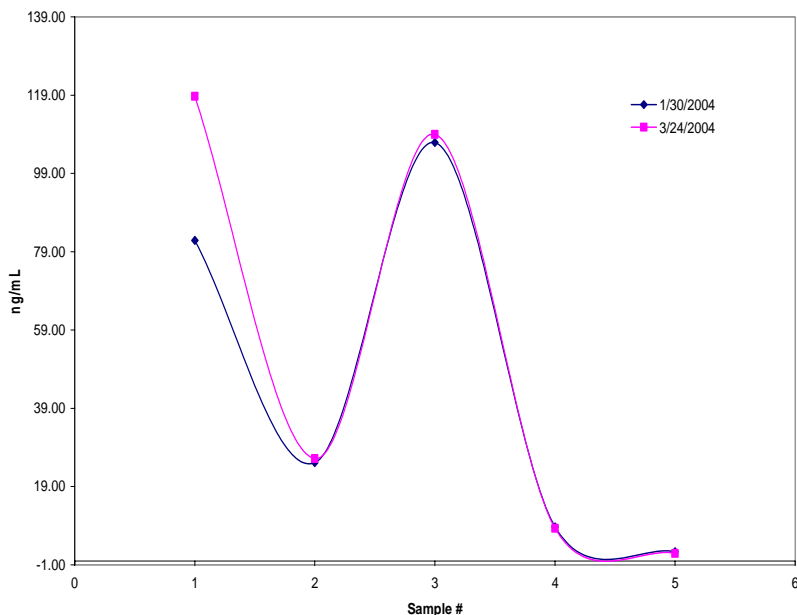
Demonstration of absence of suppression



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Infusion chromatograms demonstrating the lack of major ionization suppression or enhancement at the retention times of Flunixin (left) and Clonixin (right).

Flunixin Repeatability DecaXP After 60 days



RESULTS

Sample #	Quantum	Deca
	Mean	Mean
1	89.90	81.90
2	25.75	25.12
3	115.00	106.89
4	8.50	8.74
5	2.31	2.39

CONTROLS

ng/mL	Quantum	Deca
CONTROL	Mean	Mean
1	1.28	1.06
20	20.37	21.43
100	105.09	105.20

Duplicate analyses on Deca XP of 5 proficiency samples separated by 60 days (left). Repeatability of results for 5 proficiency samples analyzed on two different instruments (Thermo Quantum and Thermo Deca XP Plus) (right).

Measurement Uncertainty

Type A measurement uncertainty is determined for each analytical sequence using triplicate unknown determinations (n=3) and duplicate 20 ng/mL control and calibrator determinations (n=4). The mean and standard deviation for the unknown and control points are calculated. The 95% Confidence Interval is calculated by expanding the standard deviation by k=2.23.

A non-overlap criterion is employed, such that the lower 95% limit of the unknown determination must exceed the upper 95% limit of the 20 ng/mL determination to be considered to have exceeded that value.

Because other laboratories may use methods whose LLOQ is of the same order of magnitude as the 10 ng/mL threshold, this laboratory sets the threshold of reporting to be 20 ng/mL. An independent laboratory's refereed analysis is considered to be the final arbiter of any contested finding, regardless of the relative confidences of the independent determinations. Thus a benefit of the doubt is granted to ensure that borderline determinations are not overturned by referee results having dissimilar accuracies and precisions.

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Method Uncertainty

1. Control charting of the 20 ng/mL analysis mean is maintained for each analysis. Values exceeding the 95% confidence interval ($k=2$) require corrective action, including, but not limited to outlier exclusion, calibrator and control secondary stock re-preparation, investigation of pipettes used, investigation of chromatographic integrity, and possible re-preparation and re-analysis.
2. Overall method uncertainty for a specific concentration (range) can be estimated by the precision data for the range of calibrators and could be estimated for any given concentration by the power function describing the coefficient of variance across this range ($y = a X^b$ where y is CV and X is concentration). Because type A measurement uncertainty is used for reported concentration determinations, method uncertainty is used for process capability control and correction.
3. Comparison of samples run on two different mass spectrometer types yielded agreement within the range of each individual instruments inter-assay precision.

General Uncertainty Listing

Reference Standard Material - USP grade reference Flunixin Meglumine. While United States Pharmacopoeia does not provide certificates of analysis, purity of reference material may be assumed to be 99.9%.

		0.1%
Gravimetric determination-balance accuracy at 5 MG certified at 99.8%		0.2%
Gravimetric determination-precision at 5 MG determined to 99.74%		0.2614%
Volumetric Uncertainties-10 mL Class A		0.1 %
	1 mL PipetteMan	1.0 %
	0.1 mL PipetteMan	1.0 %
Sample Handling Uncertainties-internal standard method is used		0 .0%
Instrument Repeatability	Low	20%
(included within method uncertainty)	Med	10%
	Hi	3%
Method Repeatability	Low	25%
	Med	15%
	Hi	5%

Other Considerations: Preventative Actions

Spiking stocks are often prepared in solvents with denaturing properties. Addition of these solvents into plasma matrix can cause unpredictable losses of analyte due to

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protein binding and precipitation. This can be compounded by the use of variable volumes to create various concentrations. This is minimized in this SOP by preparing secondary spiking stocks in water incorporating serial dilution steps. Preparing a spiking stock for each calibrator allows the addition of the same volume to each respective plasma calibrator and control.

Ion Trap separation-in-time ion statistics do not preclude good (or acceptable) quantitative performance. Ion signal stability statistics can vary by as much as 10 to 30% per scan. This is largely offset by controlling the acquisition rate of the ion trap to produce sufficient data scans across the chromatographic peak (> 10) thus allowing good integration of that signal.

The primary source of method variability was determined to be instrument repeatability, and specifically the performance of the auto-sampler and auto-injection subassemblies. Performance of these components is determined prior to analyses by injector flushing, chromatographic back-pressure, and pre-analysis column test mix performance. Post run determinations include stability of the internal standard integration through the sequence, absence of carry-over in negative controls and blanks, and the integrity of the calibration curves obtained for pre-run and post-run calibration.

XI. IDENTIFICATION CRITERIA FOR FLUNIXIN FROM EQUINE PLASMA EXTRACTS

Identification and Confirmation of Flunixin

The qualifying diagnostic ions for identification of Flunixin are m/z 297 (M+1), 279 (BP), 264, 239.

All qualifying ions for Flunixin are present in the full scan MSMS spectrum and the retention time for the suspect sample, 20 ng/mL calibrator, and 20 ng/mL QC control agree to +/- 0.15 minutes.

All qualifying ions' abundances must agree between the suspect and calibrator spectra to within relative 30%. Interfering ions must be less than 20% absolute. Spectra may be averaged and/or subtracted.

Identification of Clonixin

The qualifying ions for the internal standard, Clonixin, are m/z 263 (M+1), 245 (BP) and 210.

XII. CRITERIA FOR FLUNIXIN QUANTIFICATION FROM EQUINE PLASMA

Determination of Flunixin

The product ion used for quantification (quantifying ion) for Flunixin is m/z 279.

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The product ion used for quantification for Clonixin (IS) is m/z 245.

Examine the reported concentration for all samples. The accuracy of concentration of QC samples should be 80% - 120% for Flunixin.

The correlation of the Flunixin calibration curve must be greater than 0.990.

A sample will be determined to have exceeded 20 ng/mL only if the lower 95% confidence limit of the suspect sample (n=3) values is greater than the upper 95% confidence limit of the 20 ng/mL calibrators and 20 ng/mL QC samples (n=4).

XIII. CRITERIA FOR REPORTING A SAMPLE POSITIVE FOR FLUNIXIN

All Blanks and Negative controls contain no quantified and reported Flunixin concentration greater than 0.2 ng/mL. The mean suspect concentration is determined to be greater than 20 ng/mL by the criterion given in section XI. And the spectral quality confirms the identity of Flunixin according to the criteria of section X.

XIV. POSITIVE SAMPLE DATA PACKET ASSEMBLY ORDER

1. SAMPLE TRANSFER SHEET (WS#32)
2. SAMPLE USAGE SHEET (FORM #7)
3. SAMPLE LIST
4. LC METHOD
5. MS METHOD
6. EXTRACTED ION CHROMATOGRAM COMPARISON
7. SPECTRA COMPARISON
8. QUANTIFICATION REPORT
9. QUANTIFICATION CALIBRATION CURVE
10. CONFIDENCE DETERMINATION REPORT
11. COLUMN TEST CHROMATOGRAM
12. COLUMN TEST SPECTRUM

Other required Documentation

In addition to the positive data packet, the following documentation is required:

Sequence list print-out that is maintained in the Sequence three ring binder
Instrument usage logbook, indicating date, project, (and maintenance log if needed)

Data packets for samples determined to be negative will contain the follow elements:

1. SAMPLE TRANSFER SHEET (WS#32)
2. SAMPLE USAGE SHEET (FORM #7)
3. QUANTIFICATION REPORT
4. UNCERTAINTY DETERMINATION

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XV. Materials, Reagents, and Formulae

A. REAGENTS

Methanol, Optima grade (Cat. No. A 454-4, Fisher Scientific.)
Acetonitrile, Optima grade (Cat. No. A 996-4, Fisher Scientific.)
Water, Optima grade (Cat. No. W7-4, Fisher Scientific.)
Methyl tert-butyl ether, HPLC grade (Cat. No. E127-4, Fisher Scientific)
Ammonium Hydroxide, Certified A.C.S. PLUS (Cat. No.A669C-21, Fisher Scientific.)
Phosphoric Acid, meets A.C.S. Specification (Cat. No. 0260-3, J.T. Baker Chemicals)
Formic Acid, SupraPur (Cat. No. 11670-1, EM Science)
Monobasic Potassium Phosphate, ACS reagent (Cat. No. P-0662, Sigma)

B. SOLUTIONS

Phosphate Buffer (saturated, pH 4.5)

Prepare a saturated solution of monobasic potassium phosphate by adding KH_2PO_4 to a liter of water while stirring until no more of monobasic Potassium phosphate will go into solution and a precipitate settles at the bottom of the container. The pH of this saturated solution should be 4.5 if it is saturated.

Let the preparation stand for a minimum of 12 hours and decant the clear upper portion of the solution into a clean reagent bottle and label.

Storage Requirements

Store at 4 °C (refrigerator) in a glass container.
Discard 3 months after date of preparation if visible flocculation is observed above the settled precipitate (Precaution: algae and bacteria growth CAN occur in saturated buffer solutions).

Phosphate Buffer (pH 3.1)

Measure 800 mL of pH 4.5 Phosphate buffer
Adjust to pH 3.1 with Phosphoric acid
Bring to final volume of 1000 mL with HPLC grade water
Mix thoroughly with stirring

Acetonitrile:Water:Formic Acid (50:50:0.1)

Add 25 mL of methanol to a liter glass container.
Add 25 mL of water. Mix.
Add 50 uL of formic acid. Mix.

Storage Requirements

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Store at room temperature in a glass container.

Formic Acid 0.1%

Add 1000 uL Formic Acid to 1000 L of Water .
Mix thoroughly. Cap, label.

Formic Acid: 2.3 mM pH 5

Add 400 uL Formic Acid to 1000 mL of Water.
Mix thoroughly. Check pH. Adjust to pH 5.0 using dropwise addition of ammonium hydroxide
Cap, label, and record pH.

Acetonitrile: 0.1% Formic Acid

Add 1000 uL Formic Acid to 1000 L of Acetonitrile
Mix thoroughly. Cap, label.

C. MATERIALS

16 x 100mm culture tubes.
16 x 125mm screw cap test tubes.
Pipettes and tips.
Vortex mixer (Scientific Industries, Inc.)
Branson Ultrasonic Water Bath, 8510 (Fisher Scientific or equivalent)
pH meter (IQ Scientific Instruments)
Sample Concentrator (Dri-Block DB-3, Techne)
IEC HN-SII Centrifuge (International Equipment Company)
Rotorack (Specie-Mix, Thermolyne)
Kimwipes
2 mL auto sampler vials
200 uL Insert (Target PP Polyspring, National Scientific Company)
15 x 45 mm, 1 x 35 mm and 28 x 57 mm VWR brand vials
Balance (Mettler AT 261 Delta range, Mettler-Toledo Inc

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