FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK

IBUPROFEN, NAPROXEN AND WARFARIN
by
SOLID PHASE EXTRACTION
and
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

PRINCIPLE

Ibuprofen and naproxen are non-steroidal anti-inflammatory drugs (NSAID), commonly prescribed as analgesics. Warfarin is an anticoagulant prescribed for patients suffering from coronary artery diseases. Since the prescribed dose is dependent upon the clotting time of the patient, there are occasions in which this would be contraindicated. A patient's medical history is therefore an important factor when scheduling such cases.

This procedure is also used to quantify ketoprofen.

This procedure is used for quantitative analysis of ibuprofen (Motrin®), naproxen (Aleve®) and a qualitative analysis of warfarin (Coumadin®). The three drugs are identified based on their retention time following separation by high performance liquid chromatography (HPLC) and by the ultra-violet (UV) spectra of the eluting peaks using a diode array detector.

Ibuprofen, naproxen and warfarin are extracted from biological specimens (blood, urine, brain, liver and gastric) using solid phase extraction. Drugs are temporarily bound to a sorbent in the solid phase cartridge as the prepared sample is poured through the column. The column is washed to remove interfering compounds, followed by the elution of the drugs from the column using an organic solvent. The eluate is evaporated and the residue containing the drugs is reconstituted with mobile phase and analyzed by HPLC. Separation of ibuprofen, naproxen and warfarin is based on their affinity and interaction with the reverse phase C-18 column and the mobile phase. The more polar drugs such as naproxen interact less with the ODS-silica and therefore elute relatively faster than less polar drugs such as ibuprofen.

SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines which are detailed in the safety manual.

REAGENTS AND MATERIALS

All reagents are HPLC grade or better.

1. **Deionized water**
2. **Ethyl Acetate**. Fisher Scientific Optima or equivalent. HPLC grade or better.
3. **Sodium Hydroxide**. Certified ACS Fisher Scientific or equivalent. FW 40.00. NaOH
4. **Sodium Hydroxide Solution, 50% w/w**. Fisher Scientific Optima or equivalent.
5. **Acetonitrile**. Fisher Scientific Optima or equivalent. HPLC grade or better.
6. **Methanol**. Fisher Scientific or equivalent. HPLC grade or better.
7. **Sodium Phosphate Monobasic**. Fisher Scientific or equivalent. FW 137.99 $NaH_2PO_4.H_20$
8. **Sodium Phosphate Dibasic**. Fisher Scientific or equivalent. FW 141.96 Na_2HPO_4
9. **O-Phosphoric Acid 85%**. Fisher Scientific or equivalent. FW 98.00. H_3PO_4
10. **Polycrom Clin II** Solid Phase Extraction Column. CEREX.
11. **System 48 Processor** connected to nitrogen source.
12. **Port Plugs for System 48 Processor**
13. **Waste Rack**, SPE Rack, Collection Tube Rack
14. **Concentrator**, connected to a nitrogen source. Zymark **TurboVap**, or equivalent.
15. **Sonicator**
16. **Vortex**
17. **Centrifuge**
18. **Vacuum Filtration Apparatus**
19. **Flurbiprofen**. (Internal standard). FW 244.3 $C_{15}H_{13}FO_2$. Sigma Chemical Company or equivalent.
20. **Ibuprofen**. FW 206.3 $C_{13}H_{18}O_2$. Sigma Chemical Company or equivalent.
21. **Naproxen**. (free base) FW 217.2 $C_{14}H_{14}O_3$. Sigma Chemical Company or equivalent.
22. **Warfarin**. FW 308.3 $C_{19}H_{16}O_4$. Abbot Laboratory or equivalent.
23. **Certified Negative Blood**. Previously found to have no interfering peaks. Freeze validated negative blood. Discard after 6 months.
24. **Certified Negative Liver**. Previously found to have no interfering peaks. Freeze validated negative liver. Discard after 6 months.
25. **Certified Negative Brain**. Previously found to have no interfering peaks. Freeze validated negative brain. Discard after 6 months.
26. **Electronic pH meter**.
27. **Laboratory glassware as required**.
28. **Nylon filter membranes,** 47 mm 0.45 µm. Agilent Technologies part number 9301-0895 or equivalent.

***Note**: It is recommended that the primary reference standards used for the preparation of controls and calibrators be obtained from different manufacturers, or that they be prepared from different lot number from the same manufacturer.*

PREPARATION OF CONTROLS

Ibuprofen, naproxen and warfarin 1000 mg/L

1. Accurately weigh 50.0 mg each of ibuprofen, naproxen and warfarin into a clean, labeled, 50 mL volumetric flask. Label volumetric flask as follows: 1000 mg/L ibuprofen, naproxen and warfarin control solution. Indicate date prepared analyst, batch number, and expiration date.
2. Add 40 mL of methanol into the 50 mL volumetric flask.
3. Mix solution by mechanical stirrer until dissolved.
4. Remove stirrer and rinse with methanol from squirt bottle. Q.S. to 50 mL mark with methanol.
5. The 1000 mg/L control solution may then be transferred into appropriately labeled headspace vials and sealed with a Teflon septum and aluminum seal. Store at 2-8^{o}C. The headspace vials will now contain the working control solution. On the vials indicate date prepared, expiration date, initials of person who prepared the solution, lot number, solvent, storage condition and bottle number with total number of bottles.
6. A qualitative control containing acetaminophen, salicylic acid, theophylline, and caffeine is extracted with each batch, to provide retention time and UV spectra to identify these compounds if present in case samples. If any of these drugs are qualitatively identified, they will be scheduled for quantitation by the appropriate method.

Note: See Acmp by SPE SOP for detailed information on materials and preparation of Control Reference Solution and spiking of the low control in blank matrix.

PREPARATION OF CALIBRATORS

Ibuprofen, naproxen and warfarin calibrator 1000 mg/L.

1. Accurately weigh 50.0 mg each of ibuprofen, naproxen and warfarin into a clean, labeled, 50 mL volumetric flask. Label volumetric flask as follows: 1000 mg/L Ibuprofen, naproxen and warfarin control solution. Indicate date prepared, analyst, batch number and expiration date
2. Add 40 mL of methanol into the 50 mL volumetric flask.
3. Mix solution by mechanical stirrer until dissolved.
4. Remove stirrer and rinse with methanol from squirt bottle. Q.S. to 50 mL mark with methanol.

5. The 1000 mg/L calibrator solution may then be transferred into appropriately labeled headspace vials and sealed with a Teflon septum and aluminum seal. Store at 2-8 °C. The headspace vials will now contain the working calibrator solution. On the vials indicate date prepared, expiration date, the initials of person who prepared the solution, lot number, solvent, storage condition and bottle number with total number of bottles.

INTERNAL STANDARD

1. Accurately weigh 50.0 mg of flurbiprofen and transfer to a 50 mL labeled volumetric flask.
2. Add 40 mL of methanol.
3. Stir with magnetic stirrer until dissolved.
4. Remove stirrer and rinse with methanol from squirt bottle. Q.S. to 50 mL mark with methanol.
5. Transfer samples to headspace vials labeled with the lot number, initials of person who prepared the solution, date prepared, expiration date, solvent, storage conditions and bottle number with total number of bottles.
6. Stable for one year. Store at 2-8 °C.

ELUTION SOLVENT

The elution solvent used is ethyl acetate

PREPARATION OF 10N SODIUM HYDROXIDE

1. Add 400 mL of 50% sodium hydroxide to a 500 mL volumetric flask.
2. Q.S to 500 mL with distilled water and mix well.
3. Transfer the solution to a storage bottle labeled with who prepared the solution, date prepared and expiration date. Stable for one year.

PHOSPHATE BUFFER, 100 MM (PH 3.0)

1. Add 1.700 g sodium phosphate monobasic to a 1 L volumetric flask.
2. Add 12.14 g sodium phosphate dibasic to the 1 L volumetric flask.
3. Dilute to 1 L mark with distilled water and mix.
4. Add 3.0 mL o-phosphoric acid 85% and mix. Measure pH with electronic pH meter
5. Adjust pH if needed with o-phosphoric acid 85%.
6. Refrigerate at 5 ^{0}C in a glass or plastic bottle. Stable for 6 months as long as pH remains stable.

MOBILE PHASE C

1. Add 2400 mL of deionized water to a 4 L graduated volumetric flask.
2. Add 13 mL of 85% phosphoric acid to the 4 L volumetric flask while mixing with a mechanical stirrer for 5 minutes.
3. Add enough 10N sodium hydroxide (approximately 17-18 mL) to increase pH to 3. Check pH with electronic pH meter.
4. Add 1500 mL of acetonitrile to the 4 L volumetric flask.
5. Stir mobile phase for 30 minutes.
6. Store the mobile phase in a 4 liter brown bottle at room temperature. Label the storage reservoir with the lot number, initials of person who prepared the solution and date prepared. Discard after 3 months. Filter before use.

RECONSTITUTION SOLUTION

1. Mix 75%, freshly filtered Mobile Phase C and 25% freshly filtered methanol.
2. Discard after two weeks.

SPECIMEN PREPARATION

Blood	1.0 mL of the undiluted specimen
Urine	1.0 mL of the undiluted specimen
Brain	1.0 mL of a 1:3 homogenate
Gastric Contents	1.0 mL of a 1:10 dilution
Liver	1.0 mL of a 1:5 homogenate

Dilution of specimens

Specimens are diluted as follows:

Brain 1:3	5.0 g of brain homogenized with 10 g of deionized water.
Liver 1:5	5.0 g of liver homogenized with 20 g of deionized water.
Gastric 1:10	2.0 mL of liquid *q.s.* to 20 mL of deionized water, or 2.0 g of a homogenized, solid specimen with 18 g of deionized water.

Note: *Use a homogenate which was prepared within two weeks. Do not use homogenates older than two weeks unless low sample size requires it. Discuss with supervisor and note in case record. The entire submitted amount of gastric contents needs to be homogenized prior to sampling.*

Note: *Homogenates of dilution factors other than 1:3 or 1:5 may be used if available. If case is suspected to have a high concentration of analyte, additional dilutions may be analyzed. Record exceptions on the sequence list.*

Note: *Record the total weight of each gastric content.*

EXTRACTION PROCEDURE

1. All reagents, samples, controls and calibrators must equilibrate to room temperature before sampling.
2. Before sampling, label all 16 x 125 mm open-top test tubes. The test tube order in the rack must correspond to the order in which samples will be pipetted and injected. Each test tube must be labeled such that the specimen type, aliquot number, laboratory number and any factors unique to a given specimen are prominently written on the test tube. Handwriting must be legible.
3. Pipet 1 mL negative validated blood, negative validated brain or negative validated liver, or deionized water (negative control for urine and gastric extractions) into a 16 x 125 mm open-top test tube. Add 2.5 µL of the working calibrator solution to the test tube. (**Calibrator I**, 2.5 mg/L). . Add 4 µL of 2500 mg/L acetaminophen/salicylic acid: 1000 mg/L theophylline/caffeine control solution. This is the 10/4 mg/L acetaminophen, salicylate, theophylline & caffene low control.
4. Pipet 1 mL negative validated blood, negative validated brain or negative validated liver, or deionized water (negative control for urine and gastric extractions) into a 16 x 125 mm open-top test tube. Add 5 µL of the working calibrator solution to the test tube. (**Calibrator II**, 5 mg/L).
5. Pipet 1 mL negative validated blood, negative validated brain or negative validated liver, or deionized water (negative control for urine and gastric extractions) into a 16 x 125 mm open-top test tube. Add 10 µL of the working calibrator solution to the test tube. (**Calibrator III,** 10 mg/L).
6. Pipet 1 mL negative validated blood, negative validated brain or negative validated liver, or deionized water (negative control for urine and gastric extractions) into a 16 x 125 mm open-top test tube. Add 25 µL of the working calibrator solution to the test tube. (**Calibrator IV,** 25 mg/L).
7. Pipet 1 mL negative validated blood, negative validated brain or negative validated liver, or deionized water (negative control for urine and gastric extractions) into a 16 x 125 mm open-top test tube. (**Negative control**).
8. A low and high matrix match quality control sample is run with each batch. Pipet 1 mL negative validated blood, negative validated brain or negative validated liver, or deionized water (negative control for urine and gastric extractions) into a 16 x 125 mm open-top test tube. Add 7.5 µL of the control working solution to a matching negative matrix. This is the 7.5 mg/L control. Add 15 µL of the control working solution to the matching negative matrix in a second test tube. This is the 15 mg/L control
9. Pipet 1 mL of specimen(s) into a properly labeled 16 x 125 mm open-top test tube.

Note: *Open specimen bottles* ***one at a time****.*

Tissue samples may be quantitated by running against blood calibrators provided matrix matched blank and controls are included and pass all QC criteria.

10. Add 12.5 µL of internal standard solution to each tube.
11. Add 6 mL of pH 3.0 100 mM phosphate buffer to each tube.
12. Mix by Vortex each tube for 15 seconds.
13. Sonicate for 20 minutes.
14. Centrifuge at ≈ 3000 rpm for 20 minutes until all sediment is at the bottom of tube.
15. Label solid phase columns. Label conical test tubes. This can be done during centrifugation.
16. Place the solid phase columns on rack, then place the whole rack on top of waste rack of nitrogen processor.
17. Decant the contents of 16 x 125 mm test tubes to the corresponding solid phase column.
18. Apply positive pressure to achieve a flow rate of ≈ 1mL/min.

Note: Use port plugs (PP-003) on unused ports for even drying.

19. Wash all columns with 1mL pH 3.0 100mM phosphate buffer.
20. Apply positive pressure to achieve a flow rate of ≈ 1mL/min.
21. Wash all columns with 1mL of deionized water.
22. Apply positive pressure to achieve a flow rate of ≈ 1mL/min.
23. Increase pressure to 35 psi to dry columns for 30 minutes.
24. Remove waste rack.
25. Place rack of conical centrifuge tubes on processor. Check to make sure that each centrifuge tube is under the corresponding solid phase column
26. Place rack of solid phase columns on top of the conical centrifuge tube rack.
27. Elute column with 2.0 mL of ethyl acetate into a corresponding conical centrifuge tube. Elute by gravity, if possible. If column does not elute or elutes slowly, use squeeze bulbs to force eluting solvent through the cartridge. In very difficult cases, reduce Nitrogen pressure to ~5 psi and use the processor to force samples through.

RECONSTITUTE SAMPLES

1. Dry in concentrator with a gentle flow of nitrogen at ≈ 25 degrees Celsius, in the Turbovap or other suitable drying apparatus.
2. Label autosampler vials indicating aliquot and toxicology number (ex: 2-YY-xxxx), specimen type, dilution, analyst and date.
3. Remove tubes once completely dry.

4. With a calibrated Eppendorf Pipet or equivalent, add 200 µL of pre-mixed mobile phase to each tube.

5. Mix contents of each tube by Vortex, at low speed, for 15 seconds. If there are significant solids suspended in the solution, centrifuge the conical tubes for 10 minutes at 3000 rpm. Transfer to an insert placed in an autosampler vial.

6. Immediately seal each vial with an aluminum seal using a crimper to avoid possible contamination from other samples. Samples may also be transferred into screw cap vials and capped immediately. Physically check that the crimped seal is tight by attempting to rotate the seal. Crimp until tight, using a new seal if necessary. Do not wait until all transfers have been made to seal the vials.

INSTRUMENTATION

Instrument #3 or #4 Agilent LC 1100 Series HPLC with Autosampler equipped with a Diode-Array Detector.

Column: Supelco Sil LC-18. 7.5 cm x 4.6 mm. 3 micron particle size.

Column Temp.: 40 °C

Integrator: Computer with Agilent Chemstation software.

HPLC Method. **Ibfcomb.m**

INSTRUMENT SETUP

Information regarding the daily maintenance and standard operation of the LC1100 can be located in the Agilent instrument manuals, the HPLC Maintenance Standard Operation Procedure and the individual method Standard Operation Procedures. For screening and quantitation of HPLC samples, the following procedure must be followed.

1. All appropriate information must be recorded on the autosampler vials. This data will be transferred to the sequence list, which will be compared with the data recorded on the autosampler vials.
2. The calibrators are injected in order of increasing concentration. A blank follows the highest calibrator.
3. Unknown samples are injected next.
4. Quality control samples are run every 10^{th} sample and as the last sample on the batch..

INSTRUMENT PRE RUN PROCEDURE

LC 1100 Instrument #3 or #4 IBF/NAPROXEN/WARFARIN Parameters

1. Pump (PV5):

Stop time	15.00 min
Post time	1.00 min
Flow	1.50 mL/min
Min. pressure	10 bar
Max. pressure	400 bar
Solvent A	75.0% (mobile phase C)
Solvent B	0.0% (Bottle B-H_2O)
Solvent C	25.0% (Methanol)
Solvent D	0.0%

2. Injector:

Injection volume	25.0 µL
Draw speed	200 µL/min

3. Mobile Phase Time Table:

The run is isocratic. The flow is 1.5 mL/min for 15 minutes run time.

4. Signals:

	Sample, Bw	Reference, Bw	[nm]
A:	215 14	550	6

5. Curve Type: Power, using 4 points: 2.5 mg/L, 5.0mg/L, 10.0 mg/L, 25.0 mg/L, forced through origin.

6. Spectrum:

Store	All
From	190 nm
To	340 nm
Step	2 nm
Threshold	1.0 mAU

TEST RUN AND SEQUENCE PREPARATION PROCEDURE

In order to ensure the instruments are in working condition, analyst is required to put a test run on the instrument. This ensures that the retention time is appropriate for all the target drugs and checks for contamination of the column (this may be observed by the peak shape in the test run, i.e. a tailing peak may indicate a contaminated column).

Click on **METHOD** and load the **IBFcomb.m** method. Click on **RUNCONTROL**, then **SAMPLE INFO**. In **SAMPLE INFO SCREEN**, verify **DATA File** path is: C:Chem\32\1\Data\. In **Prefix Subdirectory** update the **FILE NAME** to **LC3 (or 4) date of run (MMDDYY)T.** Update counter to 00001. Under **Sample Parameters** note the location of the vial and sample name of test

sample (lowest calibrator Cal 2.5 mg/L). Under **comment field** note IBF Test run. Then click on **Run Method**.

If there is more than one set of calibrators in the current batch (say a batch that requires a quantitative result in two different matrices), then the "Easy Sequence" features must be used as only the Easy Sequence works with the Sequence Queue to run consecutive separate sequences. If there is only one set of calibrators, the below sequence entry procedure may be used.

SEQUENCE PREPARATION PROCEDURE

Note: the following sequence preparation procedure is used when only one set of matrix calibrators are utilized in the sequence. See Easy Sequence Preparation SOP when there are more than one set of matrix calibrators in the sequence.

Click on **SEQUENCE** and then **SEQUENCE PARAMETER**. If the instrument is running, analysts can prepare the sequence in the Offline system.

The sequence parameters screen appears and displays the eight fields that can be modified. Usually, the sequence preparer will be concerned with three:

Operator, Subdirectory and **Sequence Comment**

1. **Prefix** field. This determines the name of the data files that will be stored. Except for special circumstances, use the instrument name and the date in the form of LC4MMDD, e.g., LC40411A. If there is more than one sequence in a day, add a serial letter after the day, e.g. LC40411A.
2. On labels to be attached to the sequence note who extracted the sequence, who created the sequence, who loaded and unloaded the sequence and who processed the sequence.
3. **Part of Method to be Run** field. "According to Runtime Checklist"
4. **Wait After Loading New Method** field. Usually zero, but may be changed if the sequence contains samples that need to be run on other methods.
5. **Post Sequence Command Macro**. Unchecked. The Shutdown Method now has the Shutdown Macro run in its Runtime Checklist.
6. **Not Ready Timeout** field. 10 minutes.
7. **Sequence Comment** field. Indicate the initials of the person performing the three steps of the analysis. It should take the form of E-XX/R-YY/S-ZZ, where E stands for extraction, R stands for reconstitution, S stands for sequence, and XX, YY and ZZ stand for the initials for the analyst performing that particular part of the analysis. Indicate the assay name, mobile phase lot number and any information specific to the batch.
8. Click on **Sequence** and then **Sequence Table**. The **Sequence Table** screen contains includes the following columns for instrument #1:

SEQ line	Vial #	Sample name	Method	Inj/ vial	Sample type	Cal level	dil	Update RF	Update RT
1	1	Cal 1 2.5 mg/L	IBFCOMB.M	1	calib	1		replace	replace
2	2	Cal 2 5.0 mg/L	IBFCOMB.M	1	calib	2		replace	replace
3	3	Cal 3 10.0 mg/L	IBFCOMB.M	1	calib	3		replace	replace
4	4	Cal 4 25.0 mg/L	IBFCOMB.M	1	calib	4		replace	replace
5	5	Blood Blank	IBFCOMB.M	1	control				
6	6	1-11-9998 hrt bld	IBFCOMB.M	1	sample				
7	7	QC 7.5 mg/L	IBFCOMB.M	1	control				
8	8	1-11-9998 fem bld	IBFCOMB.M	1	sample				
9	9	QC 15.0 mg/L	IBFCOMB.M	1	control				
10	10	1-11-9999 hrt bld	IBFCOMB.M	1	sample				
			SHUTDOWN						

For the Calibrators, all calibrators should have “Update Response Factors” set to Replace. The “Update Retention Time” field should be set to Replace for the first calibrator and Average for the remaining calibrators.

The Sample Name field should be modified according to the individual specifications for each sample. After the information of each sample is entered, type an appropriate value in the Dilution column if a dilution was made (if original concentration was used, skip the dilution field. If the sequence is the last sequence of the date, put the **SHUTDOWN** in the last Seq Line to clean up the column, followed by instrument shutdown.

Click on OK after all samples have been entered.

At this point, save the sequence into a file named LC4MMDDYY. Click on **SEQUENCE** then **SAVE SEQUENCE TEMPLATE AS**. For example, LC4102711.S or any additional sequence run in the same date in the same instrument will put a letter after the date. For example, LC4102711A.S.

Click on **SEQUENCE,** then **PRINT SEQUENCE,** highlight **SEQUENCE PARAMETERS, SAMPLE INFORMATION PART, METHOD AND INJECTION PART, CALIBRATION PART AND QUANTITATION PART.** Then click **PRINT** to print the sequence on the printer.

Affix preprinted labels for chain-of-custody (Removed by and Returned by) and steps (sequence extracted by, created by, loaded by, unloaded by and processed by). Fill out the necessary information on the stickers.

BATCH ANALYSIS

1. Place sample vials in the tray, using the printed copy of the sequence to insure that each vial is placed in the correct position and that the sample name is checked against the vial and sequence. If the person loading the samples and comparing sample name is different from the person listed in the sequence, annotate by initialing and dating the sequence.
2. Click on RUN, then click RUN SEQUENCE. Observe the first injection to insure that the system is operating correctly.
3. After the sequence is finished, indicate who removed the samples.
4. After the sequence finishes, check that all data files are successfully transferred to ECM. If the files do not transfer successfully, notify the supervisor so proper corrective action can be taken.
5. After the run finishes, the data files will be in the data subdirectory on the local chemstation, and also will be automatically transferred to the ECM Server. The files will be in the LC Location, Instrument Name Cabinet, Data Drawer and Month Folder. See data processing SOP for data processing instructions.

DATA PROCESSING

All processing and review should be performed on the Chemstation. The processed data files are then archived on the ECM.

Refer to the “Data processing” section of the SOP manual for the details on processing and review of data.

REINJECTION CRITERIA

Infrequently, analyzed samples (and very rarely sequences) may need to be reinjected for a variety of reasons. Subsequent to data review, use the criteria listed below to determine if reinjection of any sample from the sequence is necessary.

1. Poor chromatography
2. If there is an apparent carryover or UID peak
3. Requests made by the appropriate supervisor

If reinjection or other unusual actions are required, annotate this on the sequence list. Any deviation from the standard procedure must be noted, initialed, and dated. If reinjection fails, repeat analysis.

Note: *When a sample or samples are reinjected, in addition to the reinjected sample(s), reinject the blank, and the associated QC samples. This will give a qualitative result. If any of the unknowns are positive then reinject all calibrators, controls and unknowns under the modified conditions.*

ACCEPTANCE CRITERIA

Subsequent to HPLC analysis, all data is examined and reviewed according to the guidelines below.

1. A least squares regression curve is calculated by the processing method during calibration. Four calibrators (2.5 mg/L, 5 mg/L, 10 mg/L and 25 mg/L) are used to establish the calibration curve. All calibrators should quantitate at ± 20% of the weighed in target. One calibrator may be dropped if the appropriate acceptance criteria are not met. The power regression line is forced through the origin.
2. Linear regression correlation (r^2) must be at least 0.98 for each analyte. If r^2 is below 0.98, only qualitative results may be reported.
3. Internal standard (IS) response comparable to the response for the matrix calibrators (greater than ½ of the average of the calibrator IS response)
4. Positive blood controls must be within ± 20% of the weighed in target. Tissue positive controls must be within ±30% of the weighed in target.
5. All negative matrix matched controls must have no interfering peaks in the area of the target analyte.
6. Peak shape should be symmetrical and Gaussian in appearance, and the retention times of the analyte peaks are ± 2 % of the calibrator retention times.
7. Sample with final concentrations in excess of the highest calibrator must be diluted and re-extracted along with the appropriate matrix.
8. UV spectra of the analyte peaks in question must have the same absorbance curve as the equivalent peak in the respective calibrators. Samples in which co-eluting peaks (UV spectra which do not match the UV spectrum of the corresponding calibrator analyte) are detected are to be confirmed by alternate methods (i.e. TLC or GCMS). If acetaminophen, salicylic acid, caffeine and/or theophylline are presumptively detected by corresponding retention time and UV spectra match, the detection of the analyte is noted on the report and the sample scheduled for the appropriate quantitative method for the analyte.

REPORTING

1. All results must be entered on the result summary form in the case file.
2. Copies of all the calibrators and controls along with a copy of the sequence worksheet must be attached to the original chromatogram of the case, and placed in the case file folder.
3. All negative cases are reported on the result summary form as “Ibuprofen, naproxen and/or warfarin not detected”.
4. Concentrations below the lowest calibrator are reported as “Ibuprofen, naproxen and/or warfarin not detected”.
5. Concentrations greater than 2.5 mg/L are reported to one decimal place (e.g., naproxen 7.67 mg/L is reported as 7.6 mg/L).

6. When reporting gastric contents, in addition to the quantitation as mg/kg, the gastric content is also reported as mg total in the stomach content (concentration in mg/kg x weight in kg = total drug in mg).

7. Positive results must be confirmed or substantiated by either repeat analysis or by positive results based on a different analytical principle or in an additional tissue.

REFERENCES

Agilent 1090 and Agilent 1100 Series II HPLC Systems. Installation Guide.

Agilent 1090 and Agilent 1100 Series II HPLC Systems. Users Guide.

Agilent 1090 and Agilent 1100 Series II HPLC Systems. Standard Operating Procedures.

Turbovap. Users Guide.