

METHOD EVALUATION CONFIRMATION FOR OPIATES & AMPHETAMINES BY LCMS

| | |
|----------------------------|---------------------|
| Evaluation Dates: | 20/10/08 – 26/11/08 |
| Assay Implementation Date: | 1/12/2008 |

| | |
|------------------------------------|---------------|
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REASON FOR EVALUATION

The first stage of a routine drug screen is to test by immunoassay for different classes of drugs. This broad spectrum screen will detect a range of classes of drugs, but will not specifically identify them. A second, more specific method is used to confirm the presence of the drug, and also to classify the opiates or amphetamine class substances present. The existing technology is based on a TLC method that is over 30 years old, and is not suitable for today's toxicology laboratory. The purchase of an ion trap mass spectrometer this year has enabled an LCMS method to be developed which can confirm and classify a range of drugs to lower levels than the immunoassay. This will increase confidence in the results, reduce the turnaround times, increase the range of drugs that we can confirm and eventually increase the repertoire of the laboratory.

PROCEDURE DETAILS

A urine sample is spiked with a deuterated internal standard and loaded into a 2mL HPLC vial. A 100µL sample is injected directly into the HPLC system, consisting of an in-line Strata X Solid Phase Extraction cartridge, a divert valve and a 100x2.1mm 5µm PFP Gold HPLC column running a gradient of acetonitrile and water with 0.1% formic acid.

In each cycle of the analysis, a full spectrum is obtained to identify the parent masses present. These masses are compared to a mass list, and if a defined amount of a specific mass is present within a specified time window, a MS2 or MS3 scan is triggered. This MS2 & MS3 scans will produce a product ion scan of the drug, which can be compared to a standard library to confirm the presence of the drug. Up to 5 drugs can be identified from a full scan before the next full scan cycle begins. This scan cycle takes approximately 1 second, ensuring that there are normally several data points for each drug. Since there are specified time windows for each drug, a large number of drugs can be scanned for during a single analytical run.

On completion of the analysis, the MS2 spectra are compared with the library spectra, and if they give an acceptable match and the retention time is similar, the drug is determined to be present. It is also important to examine the chromatogram, as the high background readings may be interpreted as positive by the ToxID software.

SCOPE

This evaluation covers the analytes currently detected by TLC and GC, as well as 6-monoacetylmorphine. Standard solutions for the following analytes have been obtained from LGC Standards.

| | | |
|----------------------|------------------------|-----------------|
| Morphine | Codeine | Dihydrocodeine |
| Cocaine | Cocaine metabolite | Methadone |
| Methadone metabolite | Amphetamine | Methamphetamine |
| 6-monoacetylmorphine | Morphine-3-glucuronide | |

The following analytes are not available to us, as they are schedule I drugs requiring a home office licence. However, they are present in the BioRad C2 and C4 controls, and these have been used for the evaluation.

| | | |
|-----|----------------|------|
| MDA | MDMA (ecstasy) | MDEA |
|-----|----------------|------|

SAMPLE REQUIREMENTS

This method has been validated for urine samples, for which 1mL of sample is needed. Smaller volumes can be used, although the quantity of internal standard will need to be adjusted.

RISK ASSESSMENTS

WORK ACTIVITY RISK ASSESSMENT

A risk assessment for the LCMS, the HPLC and the complete procedure has already been performed. [HR-CB-TOX-LCQFLEET], [HR-CB-TOX-JASCO], [HR-CB-TOX-UDS]

COSHH RISK ASSESSMENT

| Substance | Handling requirements | Risk Rating |
|------------------|--|-------------|
| Methanol | Gloves, goggles and lab coat must be worn when handling methanol. Methanol is flammable and only small quantities should be left on the LCMS as required. | L |
| Acetonitrile | Gloves, goggles and lab coat must be worn when handling acetonitrile. Acetonitrile is flammable and only working quantities of solvent should be stored on the LCMS. Solvent must be prepared in the fume cupboard | L |
| Formic Acid | Formic acid is corrosive and gloves, goggles and lab coat must be worn when preparing the HPLC solvents. | L |
| Ammonium Formate | Irritant to eyes. Goggles must be worn when handling ammonium formate or aqueous HPLC eluant | L |
| Calibrators & QC | Treat as infectious samples. Gloves must be worn when handling these materials | L |
| Helium Cylinder | Helium is non-hazardous when used as directed. The helium cylinder is heavy and should be considered a manual handling risk. | L |

ASSESSMENT OF STAFF EXPOSURE:

RISK RATING: Low.

COMPARISON OF RESULTS

Patient sample results from the proposed method were compared with results from samples analysed by TLC for Opiates or GC for amphetamines during October and November 2008. Samples that failed the normal reflex testing rules were also analysed to increase the number of methadone/cocaine samples and to ensure that the threshold for positive opiate samples was not set too low.

All results are qualitative by LCMS, TLC and GC and so cannot be processed using a Deming regression. Direct comparison of patient results shows the following:

| | Morphine | EDDP | Codeine | Methadone | Cocaine | Amphetamine | DHC |
|-------------|----------|--------|---------|-----------|---------|-------------|--------|
| True POS | 56 | 83 | 2 | 80 | 50 | 3 | 2 |
| True NEG | 39 | 17 | 93 | 20 | 48 | 97 | 98 |
| False POS | 4 | 0 | 5 | 0 | 2 | 0 | 0 |
| False NEG | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| SENSITIVITY | 98.2% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| SPECIFICITY | 90.7% | 100.0% | 94.9% | 100.0% | 96.0% | 100.0% | 100.0% |

The results of the TLC or GC are taken as "TRUE" and LCMS results are compared to these.

$$\text{Sensitivity} = \frac{\text{LCMS POSITIVES}}{\text{TLC POSITIVES}} \quad \text{or} \quad \frac{\text{True Positives}}{(\text{True POS} + \text{False NEG})}$$

$$\text{Specificity} = \frac{\text{LCMS NEGATIVES}}{\text{TLC NEGATIVES}} \quad \text{or} \quad \frac{\text{True Negatives}}{(\text{True NEG} + \text{False POS})}$$

The anomalous results have been investigated, with the following results.

False Positive cocaines

These 2 samples had cocaine results of 204 and 238 by immunoassay. Cocaine IS present at LCMS detectable (but below cutoff) levels. This would be reported as Negative by immunoassay.

False Positive Codeines

Codeine is a common contaminant of heroin, and the presence of codeine should not be unexpected. The limit of detection by LCMS is much lower than by TLC, and in four of these samples, morphine (from heroin) was present. In the fifth sample, opiates were detected by immunoassay, but neither morphine nor codeine were detected by TLC.

False Positive Morphines

Three of these samples were opiate immunoassay positive, but no morphine was detected by TLC. The detection limit for morphine is slightly less for LCMS than TLC, but the level of interference is lower, enabling more certain identification of morphine.

The fourth sample was codeine positive, with only a weak morphine concentration. In most of the UK population, codeine can be metabolised to morphine, and this probably represents codeine use rather than heroin.

False Negative Morphine

This sample has been re-analysed by TLC and found to have no morphine. There is significant interference covering the morphine area on the plate, and the original identification of a weak positive morphine was uncertain and made in conjunction with the patient history. This shows how the added certainty of identification by spectral library matching gives improved results compared to the existing method.

INTRA ASSAY PRECISION

Intra assay precision was measured at the three control levels for each analyte. Since there is no calibration and there are insufficient data points for quantitation, this can only give some idea of reproducibility of peak intensities. Peak intensities were found to have good precision even below the cut-off limit.

The peak intensities that relate to cutoff concentrations of the drugs are shown below. These intensities will vary slightly between runs, and are adjusted with the cutoff calibrator. Results are reported as positive only if immunoassay positive.

| Analyte | Immunoassay Cutoff (ng/mL) | LCMS Cutoff (ng/mL) | Peak Intensity (counts/s) |
|--------------------|----------------------------|---------------------|---------------------------|
| Morphine | 1000 (Opiates) | 500 | 150 |
| Codeine | 1000 (Opiates) | 500 | 100 |
| Methadone | 300 | 250 | 12500 |
| EDDP | 100 | 100 | 600 |
| Cocaine metabolite | 300 | 125 | 300 |
| Amphetamine | 500 (Amphetamines) | 250 | 80 |
| MDMA | 500 (Amphetamines) | 250 | 1000 |

| Morphine | Level C2 Positive | Level C3 Negative | Level C4 Positive |
|----------|-------------------|-------------------|-------------------|
| 1 | 577 | 95 | 935 |
| 2 | 635 | 137 | 1410 |
| 3 | 698 | 162 | 1534 |
| 4 | 563 | 136 | 1417 |
| 5 | 594 | 90 | 1479 |
| SD | 54.5 | 30.6 | 240.1 |
| Mean | 613 | 124 | 1355 |
| %CV | 8.9% | 24.7% | 17.7% |

| Codeine | Level C2 Positive | Level C3 Negative | Level C4 Positive |
|---------|-------------------|-------------------|-------------------|
| 1 | 237 | ND | 663 |
| 2 | 228 | 55 | 521 |
| 3 | 237 | ND | 568 |
| 4 | 207 | 53 | 629 |
| 5 | 248 | 70 | 487 |
| SD | 15.4 | 9.3 | 73.1 |
| Mean | 231 | 59.3 | 574 |
| %CV | 6.6% | 15.7% | 12.7% |

| Amphetamine | Level C2 Negative | Level C3 Positive | Level C4 Positive |
|-------------|----------------------|----------------------|----------------------|
| 1 | 65 | 122 | 263 |
| 2 | 68 | 101 | 286 |
| 3 | 68 | 104 | 250 |
| 4 | 65 | 94 | 269 |
| 5 | 65 | 91 | 275 |
| SD | 1.6 | 12.1 | 13.4 |
| Mean | 66.2 | 102.4 | 269 |
| %CV | 2.5% | 11.9% | 5.0% |

| Cocaine metabolite | Level C2 Negative | Level C3 Positive | Level C4 Positive |
|-----------------------|----------------------|----------------------|----------------------|
| 1 | 216 | 382 | 1442 |
| 2 | 234 | 343 | 1452 |
| 3 | 193 | 373 | 1330 |
| 4 | 169 | 373 | 1315 |
| 5 | 206 | 414 | 1373 |
| SD | 24.5 | 25.4 | 62.8 |
| Mean | 204 | 377 | 1382 |
| %CV | 12.0% | 6.7% | 4.5% |

| Methadone | Level C2 Negative | Level C3 Positive | Level C4 Positive |
|-----------|----------------------|----------------------|----------------------|
| 1 | 11660 | 15009 | 26879 |
| 2 | 11327 | 13804 | 26944 |
| 3 | 11501 | 14647 | 27245 |
| 4 | 11629 | 15235 | 25901 |
| 5 | 11033 | 15137 | 26553 |
| SD | 257.76 | 582.3 | 512.0 |
| Mean | 11430 | 14766 | 26704 |
| %CV | 2.3% | 3.9% | 1.9% |

INTER ASSAY PRECISION

Inter assay precision was measured at three levels for each analyte:

| Morphine | Level C2 Positive | Level C3 Negative | Level C4 Positive |
|----------|----------------------|----------------------|----------------------|
| 1 | 577 | 95 | 935 |
| 2 | 604 | 102 | 1417 |
| 3 | 652 | 92 | 1324 |
| 4 | 632 | 104 | 1209 |
| 5 | 592 | 99 | 1389 |
| SD | 30.4 | 4.9 | 195.9 |
| Mean | 611.4 | 98.4 | 1254.8 |
| %CV | 4.97% | 5.01% | 15.61% |

| Codeine | Level C2 Positive | Level C3 Negative | Level C4 Positive |
|---------|-------------------|-------------------|-------------------|
| 1 | 228 | 55 | 521 |
| 2 | 241 | 40 | 367 |
| 3 | 235 | 43 | 629 |
| 4 | 221 | 52 | 489 |
| 5 | 246 | 54 | 542 |
| SD | 10.0 | 6.8 | 95.1 |
| Mean | 234.2 | 48.8 | 509.6 |
| %CV | 4.26% | 14.00% | 18.67% |

| Amphetamine | Level C2 Negative | Level C3 Positive | Level C4 Positive |
|-------------|-------------------|-------------------|-------------------|
| 1 | 65 | 122 | 263 |
| 2 | 52 | 134 | 322 |
| 3 | 43 | 137 | 269 |
| 4 | 54 | 143 | 289 |
| 5 | 61 | 145 | 302 |
| SD | 8.5 | 9.1 | 24.2 |
| Mean | 55 | 136.2 | 289 |
| %CV | 15.48% | 6.68% | 8.36% |

| Cocaine | Level C2 Negative | Level C3 Positive | Level C4 Positive |
|---------|-------------------|-------------------|-------------------|
| 1 | 216 | 382 | 1442 |
| 2 | 238 | 431 | 1315 |
| 3 | 212 | 412 | 1194 |
| 4 | 187 | 379 | 1093 |
| 5 | 232 | 424 | 1254 |
| SD | 19.9 | 23.9 | 130.8 |
| Mean | 217 | 405.6 | 1259.6 |
| %CV | 9.19% | 5.90% | 10.38% |

| Methadone | Level C2 Negative | Level C3 Positive | Level C4 Positive |
|-----------|-------------------|-------------------|-------------------|
| 1 | 11660 | 15009 | 26879 |
| 2 | 11444 | 13059 | 22100 |
| 3 | 12876 | 13548 | 25901 |
| 4 | 11413 | 14863 | 18080 |
| 5 | 10942 | 14354 | 23408 |
| SD | 724.8 | 842.5 | 3473.9 |
| Mean | 11667 | 14166.6 | 23273.6 |
| %CV | 6.21% | 5.95% | 14.93% |

LINEARITY

Since results are reported qualitatively, linearity is not an important measure. Any result above the cutoff is reported as positive, and the LCMS has Automatic Gain Control to limit any overloading effects. If too many ions enter the trap, the mass resolution of the trap is reduced. Automatic Gain Control monitors the ions entering the ion trap and prevents further ions entering once the maximum value has been reached. Scan time will be reduced, but the resolution and counts per second will be maintained.

INTERFERENCE STUDIES

The combination of chromatography and spectrum matching should remove most interferences. Where possible, spectra have been produced using a collision energy optimised to retain the parent ion at a lower intensity than the most intense daughter ion. MS3 spectra are also used to increase the specificity of detected analytes.

One of the most significant sources of interference in LCMS is ion suppression. Constant infusion of a marker compound (methaqualone) post-column as a patient sample is injected can be used to identify ion suppression. Since the SPE cartridge removes most of the interferences, ion suppression studies show a maximum 20% ion suppression during the analytical run. This has negligible effect on the detection of drug analytes and is judged not to be significant.

The analytes that cause the greatest problems with the existing techniques are Quinine (cocaine interference), mebeverine (amphetamine interference) and codeine/dihydrocodeine ambiguity. No interference was seen, and codeine, dihydrocodeine and quinine are specifically identified.

DETECTION AND QUANTITATION LIMITS

Limits of detection are below the levels required to report a drug as Positive. A cut-off calibrator has been prepared to define the signal intensity that is regarded as positive, and the calibrator values are generally $\frac{1}{2}$ that required to give a positive immunoassay result. This should ensure that all positive results are confirmed by LCMS, and that non-confirmed results only occur where there is immunoassay cross reactivity.

The limit of detection of 6-monoacetylmorphine is around 25ng/mL. A lower result can be detected in full scan mode, but this should not normally be needed. The NEQAS threshold for 6-MAM is set at 10ng/mL, so it will be important to monitor Heathcontrol samples to ensure that there are no false negative results.

CARRY OVER/CARRY UNDER

Patient samples with low and high values were analysed in the following order: LLLHHLLL. The mean of the three results was calculated and the individual differences from the mean calculated. Where carry over is present, the first result for the second set of the low samples will be higher than for those following, and higher than the mean for the first set.

Where carry under is present, the first result for the high samples will be lower than those of the following high samples.

| Morphine | Result | Mean | % Diff |
|----------|--------|------|--------|
| L | 0 | 0 | 0 |
| L | 0 | 0 | 0 |
| L | 0 | 0 | 0 |
| H | 333 | 362 | -8.0% |
| H | 383 | 362 | 5.8% |
| H | 370 | 362 | 2.2% |
| L | 0 | 0 | 0 |
| L | 0 | 0 | 0 |
| L | 0 | 0 | 0 |

| Methadone | Result | Mean | % Diff |
|-----------|--------|-------|--------|
| L | 37.7 | 38.3 | -1.7% |
| L | 38.5 | 38.3 | 0.4% |
| L | 38.8 | 38.3 | 1.2% |
| H | 28100 | 28967 | -3.0% |
| H | 27900 | 28967 | -3.7% |
| H | 30900 | 28967 | 6.7% |
| L | 171 | 112.6 | 51.9% |
| L | 102 | 112.6 | -9.4% |
| L | 64.7 | 112.6 | -42.5% |

| Cocaine metabolite | Result | Mean | % Diff |
|--------------------|--------|-------|--------|
| L | 73.4 | 120.1 | -38.9% |
| L | 145 | 120.1 | 20.7% |
| L | 142 | 120.1 | 18.2% |
| H | 1230 | 1120 | 9.8% |
| H | 1060 | 1120 | -5.4% |
| H | 1070 | 1120 | -4.5% |
| L | 127 | 150.3 | -15.5% |
| L | 173 | 150.3 | 15.1% |
| L | 151 | 150.3 | 0.4% |

| Methadone metabolite | Result | Mean | % Diff |
|----------------------|--------|------|--------|
| L | 32.3 | 35.1 | -8.1% |
| L | 35.6 | 35.1 | 1.3% |
| L | 37.5 | 35.1 | 6.7% |
| H | 1090 | 1100 | -0.9% |
| H | 1030 | 1100 | -6.4% |
| H | 1180 | 1100 | 7.3% |
| L | 48.2 | 51.6 | -6.6% |
| L | 52.4 | 51.6 | 1.6% |
| L | 54.2 | 51.6 | 5.0% |

Although there appears to be carry over in the methadone, the positive cutoff value is around 12000, so this carryover is insignificant. The carry-under shown is within the daily variation of the assay. No positive results were found of any drug in water or negative QC samples analysed during the evaluation.

Carry over and carry under data were acceptable.

REAGENT/SAMPLE STABILITY

HPLC solvents appear to be stable for at least 1 month, and workload should ensure that solvent throughput is faster than this. QC samples are supplied liquid stable, and each open bottle should last at least 2 weeks, or until the use by date on the pack.

Samples are stable for these analytes for at least 2 weeks when kept at 4°C and for several months when frozen. Samples can also be heated for 2 hours at 60°C or kept at room temperature for at least a week with no noticeable degradation.

QUALITY CONTROL

Three quality control samples from Bio-Rad will be used in this assay. C2 should give negative (but not zero) results for all drugs except morphine and codeine. C3 should give positive results for a more limited range of drugs and negative (non-zero) results for Morphine and Codeine. C4 should give positive results for all drugs. A blank sample and a cutoff calibrator will be analysed with each batch

EXTERNAL QUALITY ASSURANCE

3 Heathcontrol drugs of abuse samples are received quarterly for a wide range of drugs. 9 samples have been reanalyzed, 6 samples from previous distributions and the 3 samples from the current distribution.

For the drugs that we report, there were no inconsistencies between the LCMS results and the reported or spiked values. This included the following positive drugs: Morphine, methadone, EDDP, dihydrocodeine, amphetamine, methamphetamine and cocaine. There were NO false positives in these 9 samples.

REFERENCE RANGE

There is no reference range with this test. Results are reported as either Positive or Negative and compared to the patient's drug history.

STAFF TRAINING

Staff using the LCMS should be familiar with the principles of drug testing and will understand the differences between screening and confirmation assays. Training is scheduled for the week before the go-live date, and Richard and Tracey will also be available for further training until Christmas. Beyond this, it is expected that training will be through routine rotational training.

COSTS

Bottom up costs suggest that the cost of a batch of 20 samples will be approximately £5.23. The current workload is around 20-30 samples/day. This is based on a new HPLC column and SPE cartridge being required every month. These are conservative figures, and an HPLC Column will probably last significantly longer.

The current bottom up cost of opiate confirmations is approximately £5.10, suggesting that no change in pricing is required.

A full drug screen, including confirmation, currently costs £20. LCMS only has not been offered, but a price of £12/sample would give an appropriate margin.

COMPUTER CHANGES

The existing test code (OPIA_C) will be kept, but the detail tests will change. TLC will be replaced by LCMS and the GC detail will be lost. Additionally, 6MAM will be included within the group and amphetamine confirmations (AMPH_C) will be combined into the same test code. The Workcentre will remain 538 (Toxicology) testing Site 2 and results will be sent to queue 17 for authorisation. These changes must wait until the "go live" date, but have been tested in CERT.

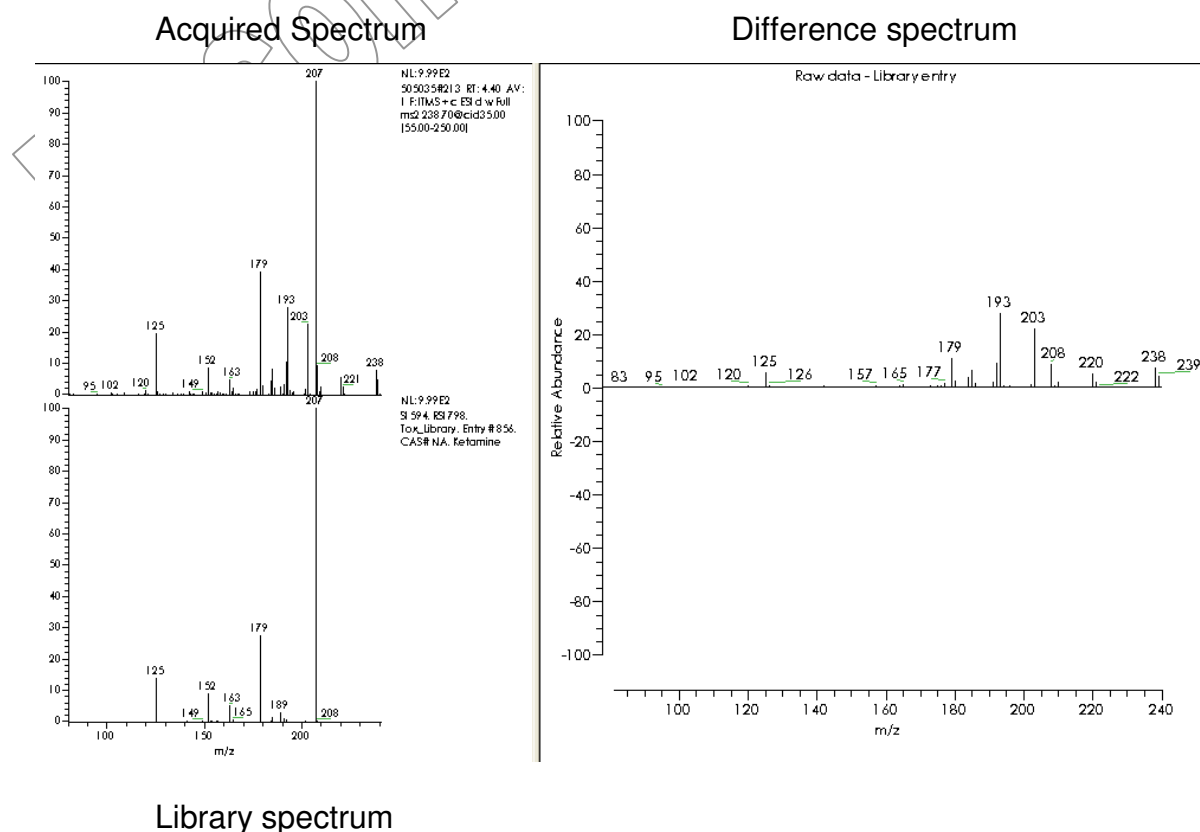
IDENTIFICATION OF OTHER DRUGS

The ion trap mass spectrometer can be used to identify unknown drugs in patient samples. A full formal evaluation may not be appropriate for all drugs, but it is important that an evaluation is performed to ensure correct sample identification. It is intended that the following guidelines are used when identifying other drugs.

A full evaluation must be done for drugs that are to be reported in all patients (eg cannabis, benzodiazepines). For less common drugs, one of the following cases must apply:

- If a drug is easily obtainable commercially, this is analysed as a patient sample and a library spectrum and retention time are recorded.
- If a drug or metabolite is not easily obtainable, but there is a sample that is known positive for this drug (eg control, NEQAS sample, patient known to be taking drug), the drug should be identified from a clear peak in an extracted ion chromatogram (XIC), together with an MS2 spectrum that is a good match with a stored library spectrum. The spectrum should also be examined by eye to ensure that it is correct.
- Where there is no known positive sample as a comparison, an identification may be made with a clear XIC peak (S/N >10) **and** a very good library match (SI >800 & RSI >900) **and** a >90% probability that this is the correct identification (from the library search window). There should also be some other supporting evidence, such as suspicion of use, an MS3 spectrum matching a theoretical or published spectrum or a similar retention time to a related compound. It is NOT intended that this procedure should be used for routine “unknown drug screening”.

As an example of this process, a sample was analysed by City Hospital in Birmingham for Ketamine, and was reported as Positive. Analysis by ion trap gave a good library match (SI=594, RSI=798) as shown below:



NEW/ALTERED PROCEDURE ACCEPTANCE FORM

Please ensure the procedure acceptance form is fully completed and signed.

Procedure Title: Opiate / Amphetamine Confirmation

Brief description of procedure:

Urine samples are extracted by online SPE and drugs separated by HPLC. Detection is by ion-trap MS with spectral library matching of drugs.

Is this procedure:

- a new assay? new equipment? a modified procedure?

Evaluation Checklist

Tick the boxes when the following have been completed or are not applicable (N/A):

- | Yes | N/A | |
|-------------------------------------|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Work activity risk assessment |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | COSHH risk assessments |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Comparison of results |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Intra assay imprecision |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Inter assay imprecision |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | Linearity |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Interference studies (list): Ion suppression, mebeverine and quinine |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Sensitivity |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Carry over |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Reagent stability |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | Sample stability |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | External Quality Assurance samples (name scheme): Heathcontrol Drugs of Abuse scheme |

Tick the boxes below when the following have been completed:

- | | | |
|---|---|---|
| <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> N/A | Reference range established |
| <input checked="" type="checkbox"/> | | Procedure/ instructions written, tested, accepted |
| <input checked="" type="checkbox"/> | | Staff trained |
| <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> N/A | Participation in External Quality Assurance scheme arranged (name): Heathcontrol Drugs of Abuse Scheme |
| <input checked="" type="checkbox"/> | | Computer changes completed |
| <input checked="" type="checkbox"/> | | Changes made to other laboratory documentation (list): SOP Instrument SOP Training Logs |

Note any comments related to the new procedure (which are not covered above) below:

New Procedure Acceptance

Procedure Title: Opiate and Amphetamine confirmations by LCMS

Name of person introducing new procedure:

Richard Evers

Grade :

Senior Toxicologist

Date:

Signature:.....

Date Ratified at Executive meeting:

Proposed start date for new procedure:

Letter sent to clinicians? Yes Not required

APPENDIX 1
(1=POSITIVE, 0=Negative)

| LAB NO: | LCMS | | | | | | | TLC | | | | | | |
|---------|-------|------|-----|------|-----|-----|-----|-------|------|-----|------|-----|-----|-----|
| | Morph | EDDP | Cod | Meth | Coc | Amp | DHC | Morph | EDDP | Cod | Meth | Coc | Amp | DHC |
| 237254 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 247634 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 272152 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 275581 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 313706 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 323758 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 327487 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 330287 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 330291 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 348721 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 348727 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 363277 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 363345 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 369726 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 369730 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 369779 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 372278 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 391832 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 391834 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 394197 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 405079 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 407349 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 411097 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 419098 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 426249 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 426254 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 432478 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 432484 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 432487 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 449093 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 449097 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 452297 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 452306 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 455254 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 456996 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 471029 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 473569 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 482765 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 485336 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 492036 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 511074 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 511102 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 522106 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 525407 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 548803 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 554484 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 556669 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 700697 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 704097 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 708790 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 715808 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 56529 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 56536 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 56540 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 562853 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 562873 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 562874 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 733109 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 733115 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 733120 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| 758298 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 758312 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 759666 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 759831 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 759838 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 759840 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |

| | | | | | | | | | | | | | | |
|--------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 759853 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 759859 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 759862 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 761173 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| 761199 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 761212 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 761219 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 761223 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 761248 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 761541 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 762753 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 762766 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 762769 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 762788 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 762804 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 762812 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 762827 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 762841 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 762894 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 762915 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 763737 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 763763 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 763772 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| 763802 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 763823 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 763847 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 765258 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 765263 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 765267 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 765269 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 765274 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 765726 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| 766980 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |

NEQAS results

| Lab No | LCMS | | | | | | | Report | | | | | | | Comparison |
|--------|-------|------|-----|------|-----|-------|-----|--------|------|-----|------|-----|-------|-----|------------|
| | Morph | EDDP | Cod | Meth | Coc | Amp | DHC | Morph | EDDP | Cod | Meth | Coc | Amp | DHC | |
| 733120 | POS | NEG | NEG | NEG | POS | POS | NEG | POS | NEG | NEG | NEG | POS | POS | NEG | match |
| 733115 | NEG | POS | NEG | POS | POS | NEG | NEG | NEG | POS | NEG | POS | NEG | NEG | NEG | match |
| 733109 | NEG | POS | NEG | NEG | NEG | NEG | NEG | NEG | POS | NEG | NEG | NEG | NEG | NEG | match |
| 056529 | NEG | NEG | NEG | NEG | POS | NEG | NEG | NEG | NEG | NEG | NEG | POS | NEG | NEG | match |
| 056536 | NEG | POS | NEG | POS | NEG | NEG | NEG | NEG | POS | NEG | POS | NEG | NEG | NEG | match |
| 056540 | NEG | NEG | NEG | NEG | NEG | NEG | POS | NEG | NEG | NEG | NEG | NEG | NEG | POS | match |
| 562853 | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | match |
| 562874 | NEG | NEG | NEG | NEG | NEG | MeAmp | NEG | NEG | NEG | NEG | NEG | NEG | MeAmp | NEG | match |
| 562873 | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | NEG | match |

(+wp coc)

