# 102. FSA-specific requirements for DTN 102 Toxicology: analysis for drugs in relation to s5A of the Road Traffic Act 1988

## 102.1 Scope

102.1.1 This section establishes the requirements for, and a common approach to, the analysis and reporting of the concentrations of certain drugs in relation to FSA – DTN 102: Toxicology: analysis for drugs in relation to s5A of the Road Traffic Act 1988 which sets the compliance requirements for analysis of whole blood and/or urine samples for the detection of drugs in relation to offences under s5A of the Road Traffic Act 1988 ('drug driving') [1].

102.1.2 Although s5A of the Road Traffic Act 1988 (s5A) [1] refers to both whole blood and urine samples, these requirements only apply to the analysis of whole blood samples, as the specified limits relate to whole blood concentrations [2].

## 102.2 Terminology

102.3 The analytical method is required to establish the presence or absence of a specified controlled drug above or below a specified limit in a sample as the arithmetic mean of the result of a number of analyses. To ensure clarity, the term 'standard deviation' (SD) shall be defined as the standard deviation derived using the results of the individual analyses or on the basis of reporting individual analyses.

## 102.4 Sample storage

102.4.1 The whole blood concentrations of the drugs covered by the s5A offence [2] may be subject to degradation over time. The forensic unit shall use storage methods which demonstrably minimise such degradation.

102.4.2 The forensic unit should consider the storage of samples prior to submission and may advise whether analysis is likely to be worthwhile;

the forensic unit may also provide commissioning parties with advice as to how to store samples to maintain their integrity for analysis.

## 102.5 Requirements for analysis

102.5.1 Any forensic unit undertaking analysis of whole blood where the results may be used for a prosecution under s5A of the Road Traffic Act 1988 [1] shall meet the requirements for analysis as stipulated.

102.5.2 The need for consistency in decisions to prosecute, which led to the adoption of the common reporting threshold (CRT), see 102.7.11, does not apply to those forensic units instructed by the defence. However, there is a requirement of the criminal justice system for the work undertaken on behalf of the defence to be to appropriate quality standards and to comply with the Forensic Science Regulator's Code of Practice [3]. Therefore, forensic units instructed by the defence shall comply with this Code (see also 102.7.14)

**Environmental requirements**

102.5.3 The following environmental requirements shall be addressed:

a. Analysis for the purpose of s5A shall be conducted separately from work involving bulk drugs. This means that bulk drug cases shall not be conducted in the Toxicology laboratory or same analytical batch as s5A analysis.

b. Analysis of samples for the purpose of s5A casework shall be conducted separately, in terms of both space and analytical batch, from batches of other toxicological casework (other than s5A or s4 of the Road Traffic Act 1988 [1]) that may contain high levels of drugs (e.g. suspected overdose cases in post-mortem casework). Separation may be achieved by management of space employed to ensure the risk of contamination is minimised, by separating work in time, and carrying out appropriate environmental checks.

c. Environmental monitoring shall be conducted to determine the presence and approximate level of any drugs being tested for in relation to s5A in the laboratory in which the sample preparation and

analysis are undertaken, in particular for cocaine, amphetamine and methylamphetamine. This shall include the use of matrix blank samples. The appearance of a drug in any sample or matrix blank where that drug should not have been present will also be monitored. The presence of a drug in a solvent blank where that drug was present in the case sample analysed immediately before the solvent blank could be the result of carry over as opposed to contamination and further investigations undertaken to attempt to determine the source and the potential effect on the result.

d. Procedures must be adopted to minimise the risk of sample contamination. At a minimum this will include appropriate separation of working areas and environment control with testing such as by swabbing of work areas to confirm absence of significant contamination.

**Analytical requirements**

102.5.4 The analytical method shall, for each drug the laboratory analyses in relation to a potential s5A [108] offence, achieve the following requirements:

a. The analysis shall be specific for each drug, such that the results can be relied on as measuring the concentration of the drug.

b. The analytical method shall ensure the results can be attributed to the sample from which they are believed to come from. This will include procedures to ensure traceability as well as address the potential for carry over.

c. To protect against the risk of carry over, a solvent blank shall, subject to the following point, be run before each case sample and the results from this blank shall not show the presence of any relevant drugs. This requirement will not require a solvent blank between two case samples where they are aliquots from the same case sample.

d. The forensic unit shall calibrate the method for each batch run. A batch is the set of all samples, including calibrators, controls, blanks and case samples, that are extracted and analysed together.

e. For any part of the analysis employing a chromatographic method the forensic unit:

i. shall ensure that quality control samples (QCs) are extracted and analysed alongside and in the same way as casework samples.

ii. shall ensure the calibration curve comprises a minimum of five calibration points, not including zero, and a maximum of 20% of data points can be removed if justified due to gross error and recorded. The calibration curve shall include and encompass concentrations either side of the defined critical or cut-off concentration. The concentration range shall be appropriate for each analyte and shall encompass the critical level of interest, ideally at approximately 50-75% of the concentration range.

iii. justifiable reasons for excluding data points are a catastrophic failure resulting in: no, or insufficient, extract to inject onto the instrument; no internal standard with which to compare the analyte response; no analyte with which to compare the internal standard response. Examples include but are not restricted to tube breakage, failure to add internal standard, failure to add analyte.

iv. shall ensure that data points generated from calibrators are reviewed prior to reviewing any QCs on a batch.

v. the calibration curve shall have a coefficient of determination (R2) greater than 0.990 linear or greater than 0.995 quadratic.

vi. shall ensure manual integration of peaks is scientifically justified and applied consistently throughout a batch and recorded. Manual integration of a peak shall not be undertaken solely to ensure an ion ratio passes or to improve the calibration curve.

vii. where manual integration has been used on a case sample and this has caused the result to be reported as over the legal limit,

where this would not have happened without the use of manual integration, the sample extract shall be reinjected or sample shall be re-extracted and repeated.

f. For any part of the analysis employing a mass spectrometry method:

   i. acceptable ion ratios should be based on the World Anti-Doping Agency (WADA) guidelines [4] or OJEC [5], ASB/ANSI [5], GTFCh [6], EWDTS [7] guidelines.

   ii. If an ion ratio fails within the case sample the result shall not be reported.

   iii. If an ion ratio fails within a QC the result shall not be used.

g. A blank whole blood sample shall be run containing an internal standard on each analytical batch. This sample shall be monitored for the presence of any drugs being analysed and the forensic unit shall assess whether there is any risk to the final reported case sample results in a batch if a drug is detected.

h. The method shall involve monitoring for analytical results which suggest there may have been a contamination event (e.g. the presence of cocaine without benzoylecgonine (BZE) or drugs appearing where not expected).

i. The reported result of the method shall be the arithmetic mean of the analysis of at least two aliquots from the casework sample. There shall be at least two results generated (i.e. the extraction of at least two aliquots). The final output of the mean of a number of analyses will be used to calculate the ‘not less than’” figure (NLTF).

j. For QC samples the mean shall be calculated from the extraction of two independent aliquots. This requirement applies to those QC samples at the relevant legal limits.

k. For the mean of a number of analytical results to be acceptable, all of the analytical results (i.e. drug concentrations in any case sample, calibrator or QC) shall be in the range ±20% of the mean.

l. For each drug, the analytical method shall achieve the following:

i. It shall have a lower limit of quantification (LLOQ) at a concentration equal to or lower than half of the legal limit.

ii. It shall, subject to point (iii) below, have an upper limit of quantification (ULOQ) at a concentration of at least 25% greater than the CRT (see 102.7.11).

iii. For diazepam, flunitrazepam, lorazepam, oxazepam and temazepam (where the sample and QCs may require dilution to bring them within the calibration range), the forensic unit shall have an ULOQ appropriate to the method used.

iv. For each internal standard on a method, a minimum acceptable response shall be set. That limit shall be set such that the method is capable of reliably detecting the analytes at the lowest required concentration.

v. For each batch, a batch response mean shall be set from the mean of the internal standard responses for the whole batch. The acceptance range shall then be applied about that batch mean.

vi. The method shall have a systematic error of no more than ±20%.

m. The forensic unit shall be able to achieve the uncertainty of measurement requirements set out in 102.7.6. These requirements shall be maintained in routine work.

102.5.5 The forensic unit shall, for each drug, establish the uncertainty of measurement in a manner consistent with accepted guidance [8] [9] and accounting for all variables which may affect the results (e.g. different operators, analysis in different batches, analysis on different dates).

**Positive quality control**

102.5.6 The forensic unit shall undertake ongoing quality control monitoring using human blood spiked at a minimum of two different concentrations:

102.5.7 A QC at the specified drug-driving limit for each drug shall be run.

102.5.8 A QC spiked at more than 50% of the top calibrant shall also be run for the following drugs: cocaine, benzoylecgonine, delta-9-tetrahydrocannabinol, ketamine, methylamphetamine, methylenedioxymethamphetamine, 6-monacetylmorphine, morphine.

102.5.9 The second QC concentration for the drugs not specifically mentioned shall be appropriate to the chosen calibration range.

102.5.10 Further QCs within the calibration range beyond the minimum requirement may be included on a batch.

102.5.11 Each QC sample shall be a replicate analysis that matches the samples.

102.5.12 The results shall be monitored in an appropriate manner (such as a Shewhart Chart) and subjected to suitable statistical rules (e.g., the Westgard Rules [10]). Results above the s5A specified limit concentration for the drugs shall only be reported as valid if obtained while the method is under control.

102.5.13 The quality control monitoring shall use sufficient QC samples in each batch to ensure the reliability of results can be assured. As a minimum, forensic units shall use a level of positive QC samples of at least 10% of the samples in the batch (including all QC values), and at least two positive QC samples when the batch contains less than 20 samples.

102.5.14 A QC sample result shall be the average of the results from the analysis of two separate aliquots of control material. The aliquots may be taken from either a single spiked blood sample or from two samples of blank blood each spiked to the appropriate concentration.

102.5.15 QC sub-sample replicates shall be run together as a pair, in the same way as the samples, and not split across the batch. The quality control sample pairs should where possible be spaced evenly through the batch, during both the extraction and the analysis, being run at the beginning, end, and where possible, the middle of the batch.

102.5.16 The mean and standard deviation of each QC concentration shall be calculated during method validation from the analysis of QCs in at least 11

batches, each batch containing at least two QC samples, each QC 'sample' comprising of least two sub-sample aliquots.

102.5.17 The 'preliminary' Shewhart chart warning limits shall be set as the greater of, ±2 times the method standard deviation or ±60% of the FSREU, from the mean of the data.

102.5.18 The 'preliminary' Shewhart chart action limits shall be set as the greater of, ±3 times the method standard deviation or 90% of the FSREU, from the mean of the data.

102.5.19 These preliminary limits shall be replaced by initial limits once the data from 30 batches have been collected.

102.5.20 The 'initial' Shewhart chart warning limits shall be set at ±2 times the standard deviation, calculated from the 30 batches, from the mean of the data.

102.5.21 The 'initial' Shewhart chart action limits shall be set ±3 times the standard deviation, calculated from the 30 batches, from the mean of the data.

102.5.22 The batch sample results shall be rejected and an investigation shall be carried out, and documented, when:

a. One or more quality control sample results are outside of the action limits; a '1 x $_{3s}$' failure. Negative sample results may be accepted where permitted by the forensic unit's procedures, such as for example when the result is below the lower limit of quantitation.

b. One or more QC sample results from two consecutive batches are between the warning and action limits; a '2 x $_{2s}$' failure. In this event it is the results of the second batch which must be investigated and it may be necessary to review the results of the earlier batch. Negative sample results may be accepted where permitted by the forensic unit's procedures, such as for example when the result is below the lower limit of quantitation and other criteria have been fulfilled.

c. QC sample results from within a single batch lie outside of both the upper and lower warning limits; An 'R x $_{4s}$' failure. Negative sample results may be accepted where permitted by the forensic unit's

procedures, such as for example when the result is below the lower limit of quantitation and other criteria have been fulfilled.

102.5.23 The batch sample results shall be accepted, but an investigation shall be carried out, and documented, when one or more quality control sample results, within a single batch, are outside, on the same side, the warning limit; a '1 x $_{2s}$' warning.

102.5.24 The forensic unit may additionally use other rules for the monitoring of trends in QC data as they see fit. Such rules include, but are not limited to:

a. The batch sample results shall be accepted, but an investigation shall be carried out, and documented, when four consecutive QC results fall between 1 and 2 SD, on one side, from the mean; A 4 x $_{1s}$ warning.

b. The batch sample results shall be accepted, but an investigation shall be carried out, and documented, when 10 consecutive results lie on one side of the mean; A '10$_x$' warning.

c. The batch sample results shall be accepted, but an investigation shall be carried out, and documented, when seven consecutive QC results fall or rise; a '7$_t$' warning.

102.6 The data on the charts shall be reviewed every three months to compare the mean and standard deviation of the QC results, using t- and F- tests, with the values used to set the chart limits. The mean and action/warning limits may be adjusted if the comparison shows significant differences and there is some explanation for those changes.

102.6.1 Where the monitoring indicates the laboratory is no longer complying with the requirements in relation to uncertainty 102.5.4, work shall stop. A non-conforming work investigation shall be carried out and corrective action shall be taken to return the method to control.

102.6.2 Where a new lot of a certified reference material is introduced, it shall be compared, by experiment, against the existing certified reference material

to determine whether there might be a change in the operation of the method.

**Contamination**

102.6.3 Analysis for the purpose of s5A can involve detection and quantification of low concentrations of drugs. Further, even low levels of contamination could have an impact on a case.

102.6.4 Forensic units shall monitor for potential contamination events. Examples include, but are not limited to, drugs appearing in blanks, drugs appearing in calibrators or reference material which should not include them, and unusual results such as the presence of cocaine without its metabolite BZE.

102.6.5 Any contamination event shall be treated as non-conforming work and there shall be an appropriate investigation and action.

## 102.7 Reporting of results

**Units**

102.7.1 Results shall be reported in units of micrograms per litre (µg/L) to facilitate comparison against the legal limits and avoid any confusion. Results for drugs with a legal limit below 10 µg/L shall be rounded down and reported to one decimal place. Results for a drug with a legal limit equal to, or greater than 10 µg/L shall be rounded down and reported to integer values only.

**Calculation**

102.7.2 Where analytical results include a value above the ULOQ, the mean shall be calculated using (a) the analytical result which is below the ULOQ and (b) the ULOQ for the result which is above the ULOQ.

102.7.3 Where both analytical results are above the ULOQ, the mean shall be reported as above the ULOQ. The ULOQ shall be used for the calculation of the NLTF.

102.7.4 Where analytical results include a value below the LLOQ and above the LOD, the value should be reported as too low to report a meaningful

concentration. The forensic unit shall determine a form of words to use in such cases.

102.7.5 The result shall be reported by use of an NLTF unless all results are above the ULOQ.

102.7.6 The Forensic Science Regulator's Expanded Uncertainty (FSREU) (see table on page 13) shall be deducted from the mean of the analytical results. The final figure generated shall be rounded. For example, a sample with concentrations of amphetamine in replicate one of 315 µg/L and replicate two of 323 µg/L leading to a mean of 319 µg/L. The FSREU is 20% so the deduction would be 63.8 producing 255.2 µg/L. This would be rounded down to 255 µg/L.

102.7.7 Where both results are above the ULOQ, the normal reporting calculation as detailed above shall be carried out, but the figure should be reported as 'greater than ###'. For example, if the ULOQ for BZE is 250 µg/L and both analytical results exceed this figure, 20% should be deducted from 250, and the result reported as 'greater than 200 µg/L.

102.7.8 The results shall be interpreted on the basis that the figure as rounded is the relevant figure for comparison against the legal limit.

**Limits**

102.7.9 Where the drug is detected but the NLTF is equal to or less than the legal limit for the drug, the results may be reported as the drug present, but it shall not be reported as being over the limit.

**Analysis at the instruction of police or prosecution**

102.7.10 To justify a prosecution, the results of the method shall allow the practitioner to state those results support the proposition that the concentration of the drug was above the legal limit. To assess the extent to which the results of the method support the proposition, the uncertainty of measurement shall be accounted for.

102.7.11 The use of the FSREU gives rise to the concept of a CRT – the lowest measured concentration at which the result can be reported as being above the legal limit. The CRT for each drug is also given in the table on

page 13. A forensic unit will only report a result as above the legal limit when the reported result of the method is greater than or equal to the CRT for the relevant drug.

102.7.12 The forensic unit shall only provide a figure, which will be the NLTF referred to above, if its expanded uncertainty of measurement is equal to or less than the FSREU.

102.7.13 This Code covers the process by which the analytical result is produced and a conclusion reported as to whether the concentration of the drug in the sample was above the relevant legal limit. The use of an agreed uncertainty and resultant common minimum reporting threshold does raise some additional points:

a. Any report/ statement on an analysis shall make clear that the:

   i. determination of the NLTF used centrally set expanded uncertainty; and

   ii. forensic unit's calculated uncertainty for the analysis was no greater (worse) than the FSREU.

b. The requirements in section (a) above shall be achieved by declaring compliance to this Code, as long as the provisions of FSA-DTN-102 and these requirements are met.

**Analysis at the instruction of the defence**

102.7.14 Forensic units acting at the instruction of the defence (see also 102.5.2), using methods with high uncertainty of measurement, could have an adverse impact on the criminal justice system (e.g., by providing inaccurate or misleading results). The forensic unit's expanded uncertainty at the 99.7% coverage probability shall be less than the FSREU.

**Table: The legal limits** [2]. **FSREU and the CRT for each drug in England and Wales**

| Controlled drug | Legal limit (µg/L) | FSREU (%) | CRT (µg/L) | Date limit first established |
|---|---|---|---|---|
| Amphetamine | 250 | 20 | 314 | 14 April 2015 |
| Benzoylecgonine | 50 | 20 | 64 | 2 March 2015 |
| Clonazepam | 50 | 20 | 64 | 2 March 2015 |
| Cocaine | 10 | 35 | 17 | 2 March 2015 |
| Delta-9-tetrahydrocannabinol | 2 | 30 | 3 | 2 March 2015 |
| Diazepam | 550 | 20 | 689 | 2 March 2015 |
| Flunitrazepam | 300 | 25 | 402 | 2 March 2015 |
| Ketamine | 20 | 20 | 27 | 2 March 2015 |
| Lorazepam | 100 | 25 | 135 | 2 March 2015 |
| Lysergic acid diethylamide | 1 | 45 | 2 | 2 March 2015 |
| Methadone | 500 | 25 | 668 | 2 March 2015 |
| Methylamphetamine | 10 | 40 | 19 | 2 March 2015 |
| Methylenedioxymethamphetamine | 10 | 25 | 15 | 2 March 2015 |
| 6-Monoacetylmorphine | 5 | 35 | 8 | 2 March 2015 |
| Morphine | 80 | 25 | 108 | 2 March 2015 |
| Oxazepam | 300 | 20 | 377 | 2 March 2015 |
| Temazepam | 1000 | 20 | 1252 | 2 March 2015 |

## References


[1] “Road Traffic Act 1988,” [Online]. Available: https://www.legislation.gov.uk/ukpga/1988/52/contents. [Accessed 17 0 2024].

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[3] “Forensic Science Regulator's Code of Practice Issue 2,” [Online].

[4] World Anti-Doping Agency, “Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for Doping Control Purposes - Technical Document – TD2023IDCR”.

[5] OJEC, “Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. (2002/657/EC).”.*The Official Journal of the European Communities, 2002, L221/8..*

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