

INTRODUCTION

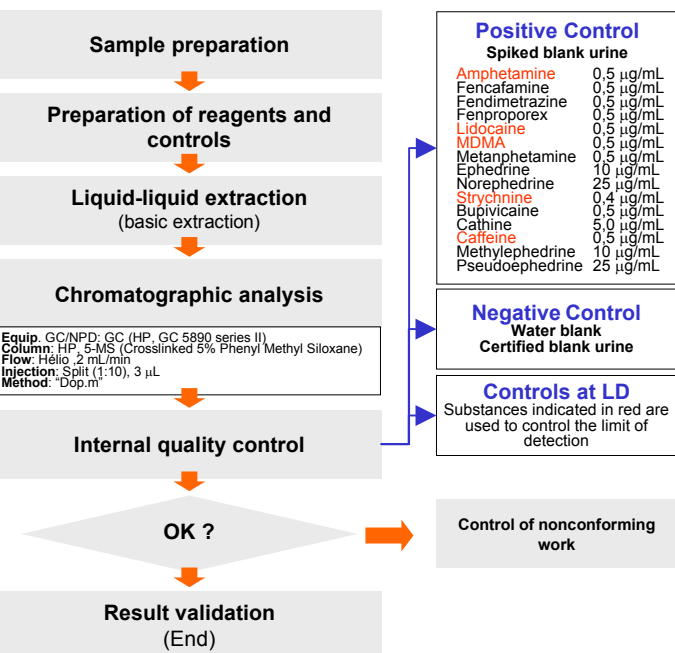
Chromatographic methods are subject to a group of variables that must be monitored and controlled in order to assure the quality of the results. In doping control analysis, the screening methods are used for the qualitative determination of a high number of substances. In this case, the most important variables include pre-analytical parameters like the extraction/hydrolysis recovery, derivatization recovery and cross-contamination, and analytical parameters (specificity, resolution, repeatability, limit of detection, among many other).

An integrated internal quality control system was developed to assure the continuous control of this type of methods. The system incorporates four essential components:

- 1 - An internal quality control methodology which incorporates a set of positive and negative controls and controls at the limit of detection.
- 2 - Four internal quality control criteria
- 3 - Three performance indicators
- 4 - A software tool that allows the calculation and automatic monitoring of all the components of the system.

This presentation exemplifies the use of this system in a screening method for volatile doping agents (stimulants, narcotics and local anaesthetics) excreted free in urine using Gas Chromatography / Nitrogen Phosphorous Detection (GC/NPD).

METHODOLOGY



Positive Control

Spiked blank urine

Amphetamine	0.5 µg/mL
Fencafamine	0.5 µg/mL
Fendimetrazine	0.5 µg/mL
Fenproporex	0.5 µg/mL
Lidocaine	0.5 µg/mL
MDMA	0.5 µg/mL
Metamphetamine	0.5 µg/mL
Ephedrine	10 µg/mL
Norephedrine	25 µg/mL
Strychnine	0.4 µg/mL
Bupivacaine	0.5 µg/mL
Cathine	5.0 µg/mL
Caffeine	0.5 µg/mL
Methylephedrine	10 µg/mL
Pseudoephedrine	25 µg/mL

Negative Control

Water blank

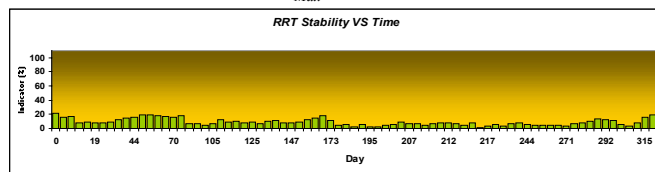
Certified blank urine

Controls at LD
Substances indicated in red are used to control the limit of detection

PERFORMANCE INDICATORS

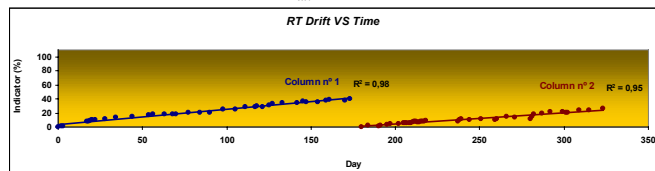
$$\text{RRT Stability} = \frac{\sum_{i=1}^n |\Delta RRT_i|}{n \times \Delta RRT_{Max}} \times 100$$

100% → Bad
0% → Excellent



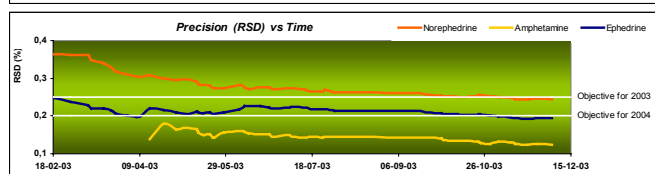
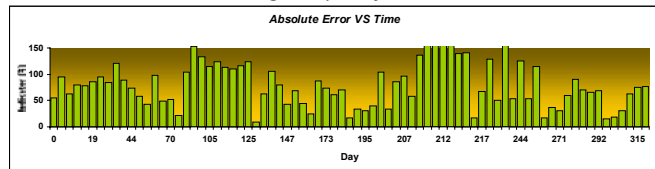
$$\text{RT Drift} = \frac{\sum_{i=1}^n |\Delta RT_i|}{n \times \Delta RT_{Max}} \times 100$$

100% → Bad
0% → Excellent



$$\text{Absolute Error} = \frac{\sum_{i=1}^n |\Delta Error_i|}{n \times \text{Quality Objective}} \times 100$$

100% → Bad
0% → Excellent



ΔRRT_i → Relative difference between RRT_i and $RRT_{i, Mean}$ (%)
 ΔRT_i → Relative difference between RT_i and $RT_{i, initial}$ (%)
 $\Delta Error_i$ → Relative difference between A/A_i and $A/A_{i, Mean}$ (%)
 A/A_i → Relative area of analyte i
 $A/A_{i, Mean}$ → Mean relative area of analyte i

ΔRRT_{Max} → 1%
 ΔRT_{Max} → 5%
 Quality Objective → 25%

QUALITY CONTROL CRITERIA

- 1 The Relative Retention Time (RRT) of each analyte in the positive control shall not differ by more than 1% from the mean Relative Retention Time (RRT_{Mean}). Additionally, all analytes must be detected with a $S/N > 3$.

This criterion allows the control of the specificity, limit of detection and precision of RRT

- 2 The absolute Retention Time (RT) of each analyte in the positive control shall not differ by more than 5% from the initial Retention Time ($RT_{Initial}$) determined when the column is used for the first time.

This criterion allows the control of the RT drift (column "ageing")

- 3 The absolute area of the internal standard must be higher than the lowest acceptable value.

This criteria control the method recovery

- 4 The relative areas of some analytes must be in-control using univariate control charts.

This criteria control the method precision

Software

A software tool was developed which allows the calculation and automatic monitoring of all the components of the system.

The software is called "Garfield".



CONCLUSIONS

- The internal quality control system resulted in a significant quality improvement by enhancing the method precision.
- The system controls all the most important parameters affecting the quality of the results.
- The RT drift is directly proportional to time. This relationship allows the prediction of the best timing to change the chromatographic column,
- The Absolute Error cannot be used as a rejection criterion because it would lead to an elevated percentage of false rejections. However, this parameter can be used as a quality indicator.

References

Barwick, V.J., Review of Sources of Uncertainty in Gas Chromatography and High Performance Liquid Chromatography, LGC/VAM (1998).