

## VALISTAT - Software for Method Validation in Forensic Toxicology

Georg Schmitt<sup>1</sup>, Michael Herbold<sup>2</sup>, Frank Peters<sup>3</sup>

<sup>1</sup>Institute of Legal and Traffic Medicine, University Hospital, Heidelberg

<sup>2</sup>ARVECON GmbH, Walldorf

<sup>3</sup>Institute of Forensic Medicine, University Hospital, Jena

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### Abstract

The special requirements for the validation of forensic-toxicological analysis were constituted by the working group 'quality assurance' of the GTFCh. For the easy execution of all necessary calculations the Excel program Valistat was developed. The following features were supported by the first version:

- examination of working range and calibration model
- examination for systematic and accidental errors
- calculation of analytical limits (DIN 32645)
- calculation of recovery (extraction yield)

According to the revised guidelines of the German Society of Toxicology and Forensic Chemistry (GTFCh) new special calculations can be performed by means of the second program version:

- processed sample stability
- evaluation of beta expectation intervals
- determination of matrix effects
- new alternative methods for the determination of the LOD and LOQ

### 1. Introduction

The special requirements for the validation of forensic-toxicological analyses are given by the guidelines of the German Society of Toxicology and Forensic Chemistry (GTFCh) [1]. These guidelines were revised in the year 2009 and valid since 1 April 2011. Accordingly, new special calculations have to be performed.

### 2. Material and Methods

For the compliance and execution of the revised guidelines the validation software (Valistat Version 2.0) was completely new developed based on the version 1.0 [2]. During the development it was the aim to make the handling of the software more simple and to arrange the layout more clearly. The program is based on Microsoft Excel 2010 but is also compatible with versions 2003 and 2007.

### 3. Results and Discussion

Due to the revision of the guidelines additional features had to be introduced. This includes processed sample stability, the calculation of the 95% beta expectation intervals as well as matrix effects. For the calculation of the LOD and LOQ alternative methods for the determination were introduced.

### 3.1. Processed sample stability

This feature determines the stability of the analytes in processed samples during the time of the analytical process (Fig. 1).

### 3.2. Beta expectation intervals

Evaluation of 95% beta expectation intervals from the systematic error (bias) and the random error (precision) and determination of its scope (target value  $\pm 30\%$  /  $\pm 40\%$  at LOQ, Fig. 2).

### 3.3. Matrix effects

Determination of the combined effect of all components of the sample on the measurement of the quantity of the analyte (Fig. 3).

### 3.4. New alternative methods for the determination of the LOD and LOQ

Calculation of the LOD using the signal to noise ratio ( $\geq 3:1$ ), calculation of the LOQ by the analysis of 5-6 replicates followed by the determination of bias ( $\pm 15\%$  of the target value) and precision ( $RSD \leq 15\%$ , Fig. 4).

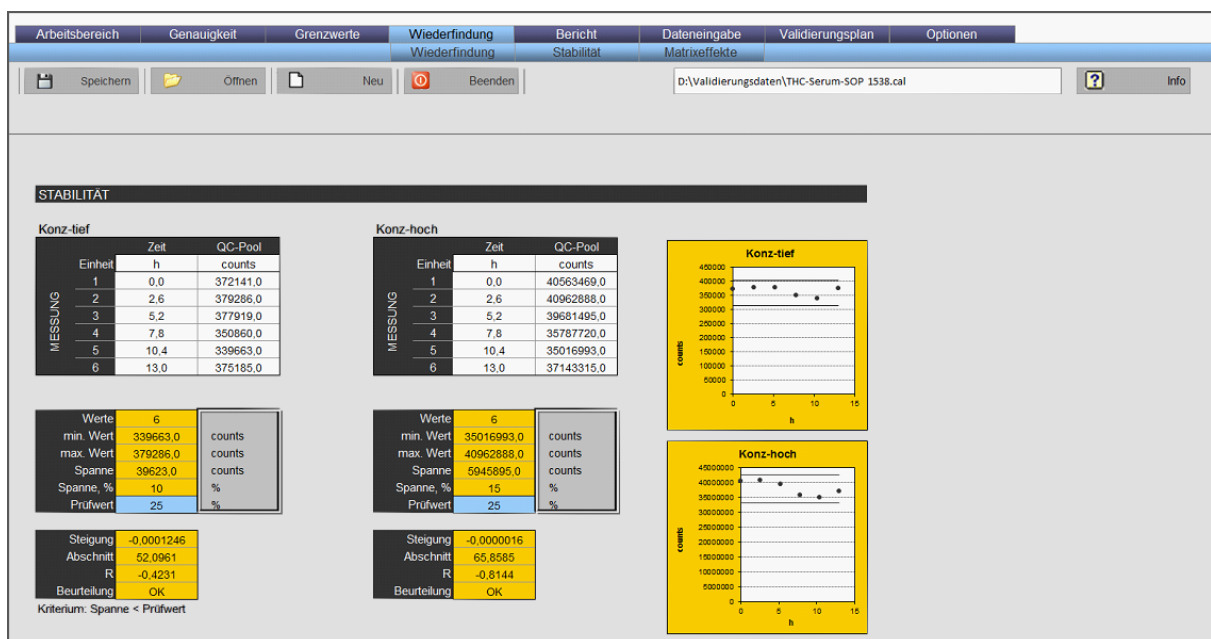


Fig. 1. Processed sample stability.

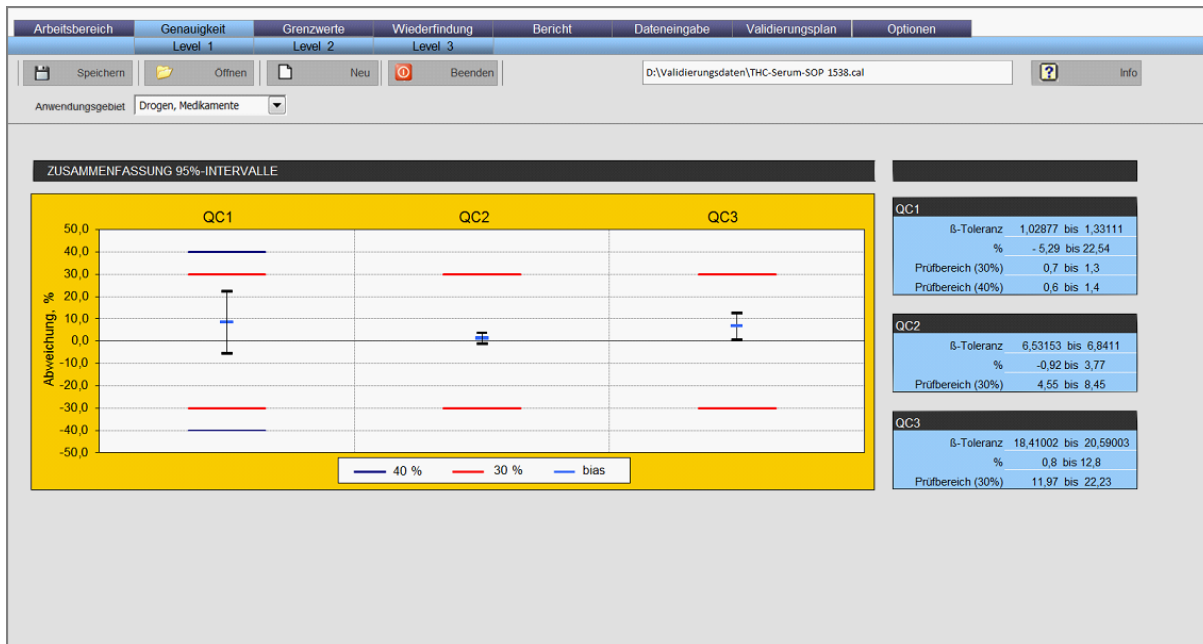


Fig. 2. Beta expectation intervals.

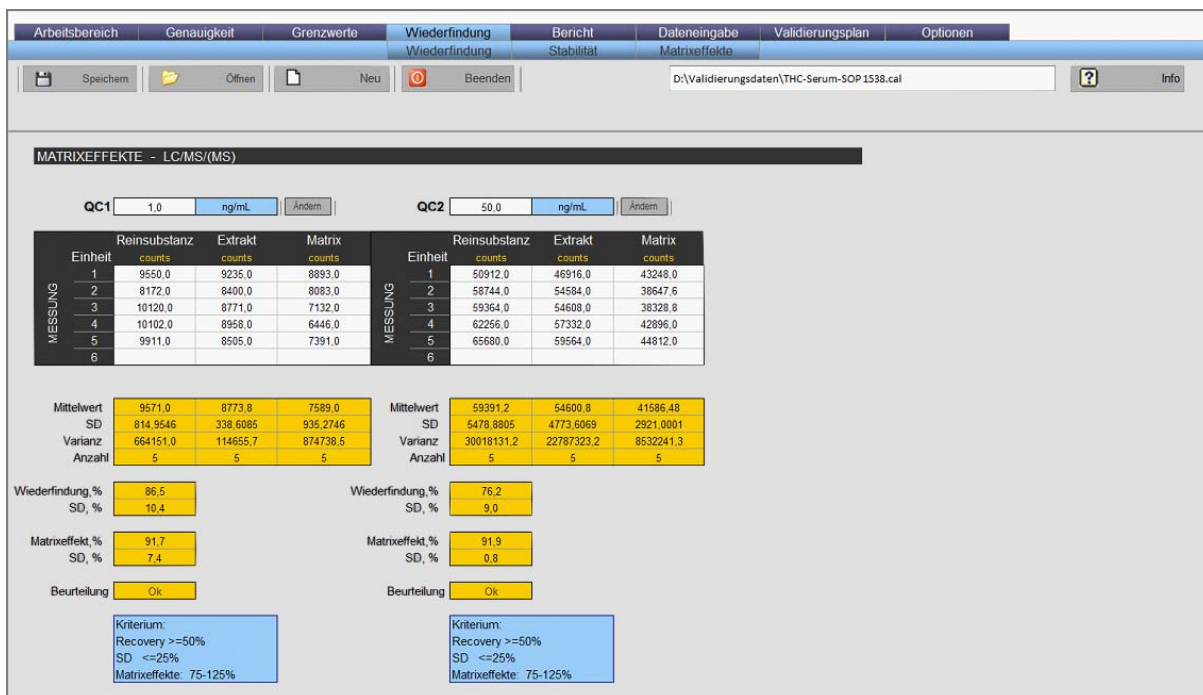


Fig. 3. Matrix effects.

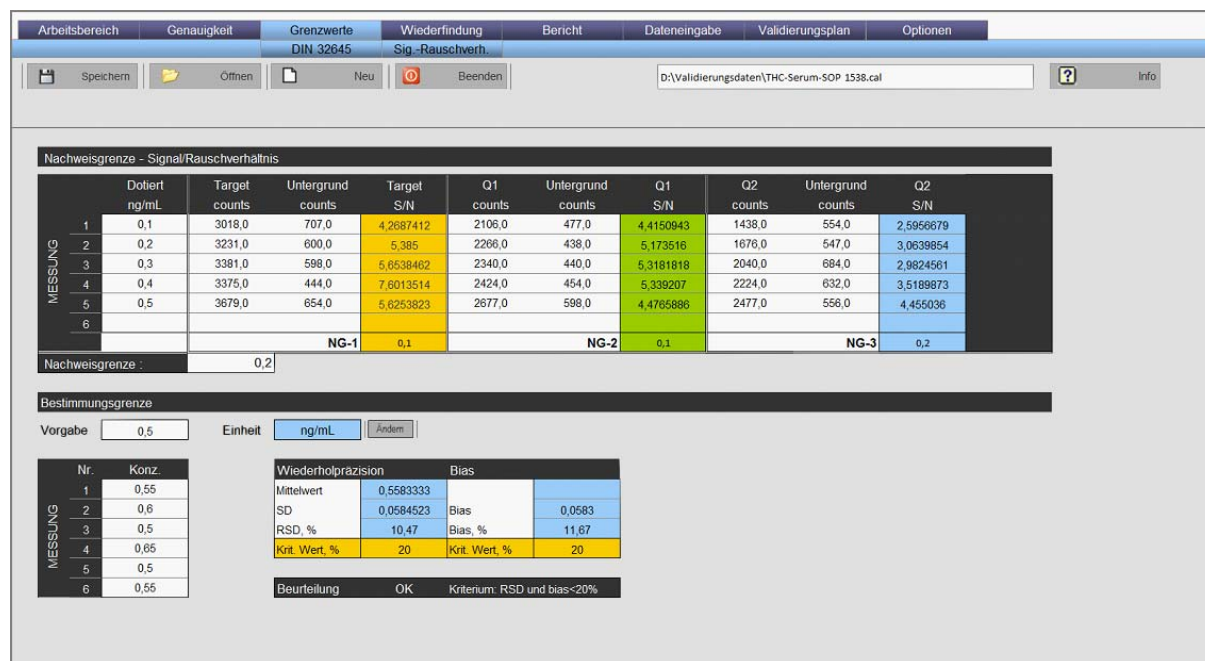


Fig. 4. New alternative methods for the determination of the LOD and LOQ.

The new requirements of the revised guidelines were included in the second version of the VALISTAT software. The following validation characteristics can be calculated: Examination of the linearity of calibration, examination for systematic (bias) and accidental errors (time-different intermediate precision), estimation of the total error using beta-expectation intervals, calculation of the sample stability, calculation of the analytical limits according to DIN 32645, calculation of the LOD using the signal to noise ratio, calculation of the recovery (extraction yield), and calculation of matrix effects. The program was tested with a special set of test data. Furthermore, the results were checked by an independent calculation. Additionally all calculations were checked and discussed within the GTFCh Workshop 2009 Heidelberg [3].

#### 4. Conclusion

The software complies with the guidelines of the GTFCh and can easily be used for the required calculations.

#### 5. References

- [1] GTFCh (2009) Guidelines of the GTFCh, Appendix B  
<http://www.gtfch.org>
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