Validation of an alcohol dehydrogenase method for forensic blood alcohol determination on Thermo Scientific Indiko analyzer and comparison with Technikon autoanalyzer

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Abstract

Aim: In context of restructuring the local forensic toxicological laboratory services a suitable and automatable method for determination of blood alcohol concentration had to be established. As alcohol dehydrogenase method the DRI-Ethanol Assay (Microgenics) on Indiko Analyzer (Thermo Scientific) was chosen. The aim of this study was to validate this method and compare the results with those of the currently used method using Technikon Autoanalyzer.

Methods: Serum or aqueous ethanol solutions were used for determination of ethanol without prior treatment and the blood alcohol concentration was measured automatically on both systems. Calculations were executed with VALISTAT.

Results: For the Indiko Analyzer, accuracy measurement with serum control samples containing 0.50 or 2.00 g ethanol/L on 9 days yielded a mean result of 0.52 +/- 0.02 g/L and 1.98 +/- 0.06 g/L, respectively. The relative standard deviation (RSD) was 3.1% (0.50 g/L) and 3.2% (2.00 g/L); bias was 3.3% and -1.2%, respectively. Repeatability was calculated with RSD = 0.00% for both controls. The intermediate precision was RSD = 2.1% (0.50 g/L) and RSD = 1.5% (2.00 g/L). These validation parameters were similar to those values obtained for the Technikon Autoanalyzer in a preceding study. The results obtained for 93 authentic forensic serum specimens measured on both systems provide a linear regression equation of y=1.0202x-0.0087 and show an excellent correlation of r=0.9977. The Limit of Detection was 0.00 g/L and the Limit of Quantification 0.01 g/L for both ADH methods.

Conclusion: Precision and reproducibility of the ADH method on the Indiko Analyzer meet the requirements for BAC determination in forensic specimens and were comparable to our currently used ADH method.

1. Introduction

In context of restructuring the local forensic toxicological laboratory service a suitable and automatable method for the determination of blood alcohol concentration (BAC) had to be established. Beside headspace gas chromatography, enzymatic methods utilizing alcohol dehydrogenase (ADH) enzyme are frequently used for the analysis of ethanol in biological specimens [1]. This method is one the methods recommended by the guidelines for the determination of forensic BAC of the Society of Toxicological and Forensic Chemistry (GTFCh) [2]. As ADH method the DRI-Ethanol Assay (Microgenics) on Indiko Analyzer (Thermo Scientific) was chosen. The aim of this study was to validate this method according to the guidelines of the GTFCh [3] and compare the results with those of the currently used method using Technikon Autoanalyzer.

2. Material and Methods

Serum or aqueous ethanol solutions were used for determination of ethanol without prior treatment. Whole blood samples were centrifuged at 2500 x g for 10 min and the supernatant (serum) was measured automatically on both systems. Calibration was carried out with aqueous ethanol standard solutions (Fa. DiaSys Diagnostic Systems). Results obtained from this calibration can be directly converted to the unit g/L ethanol in serum. Conversion to g ethanol/kg blood was done by dividing the values by 1.236. Calculations for the validation parameters were executed with VALISTAT [4] according to Schmitt & Aderjan [5].

3. Results and Discussion

3.1. Instrument Range and Calibration Range

To check the linearity of the calibration range we measured the blank value (H_2O) and the aqueous ethanol calibrators on the Indiko Analyzer multiple times. Values for extinction and the validation data are given in figure 1. The data show no outliers (Grubbs-test) and the variances between lowest and highest calibrator are homogenous (Cochran-test). Since we used a point to point calibration to cover the entire range from 0 to 5 g/L ethanol, the method did not pass the linearity test (Mandel-F-test). Comparable validation data were obtained with the Technikon Autoanalyzer (data not shown).

TARGET	Extinktion	Ändern						
Konzentration	0,0	0,2	0,5	1,0	2,0	3,0	4,0	5,0
MESSUNG 1	-0,0011	0,0172	0,0451	0,0984	0,1744	0,2750	0,3393	0,4260
MESSUNG 2	-0,0013	0,0172	0,0445	0,0952	0,1785	0,2664	0,3491	0,4208
MESSUNG 3	-0,0013	0,0171	0,0464	0,0913	0,1852	0,2620	0,3590	0,4167
MESSUNG 4	-0,0012	0,0168	0,0471	0,0911	0,1892	0,2572	0,3514	0,4212
MESSUNG 5	-0,0011	0,0167	0,0456	0,0889	0,1856	0,2582	0,3451	0,4195
MESSUNG 6	-0,0012	0,0162	0,0452	0,0873	0,1778	0,2590	0,3400	0,4192
Auswertung								
Mittelwert	-0,0012	0,0168517	0,045635	0,0920283	0,1817867	0,262935	0,3473333	0,4205733
SD	0,0000888	0,0003995	0,0009337	0,004118	0,0057009	0,00676	0,0074684	0,0031192
Varianz	0,0	0,0	0,0	0,0	0,0	0,0	0,0001	0,0
Werte	6	6	6	6	6	6	6	6
Ausreisser-Test nach Grubbs								
Extremwert	-0,0013	0,01617	0,04708	0,09844	0,1892	0,27495	0,35901	0,42603
Prüfwert	1,1265148	1,7062118	1,5476469	1,5569868	1,3003834	1,7773546	1,5634799	1,7493965
Signifikanz 95%								
Tabellenwert	1,822	1,822	1,822	1,822	1,822	1,822	1,822	1,822
Straggler?	nein	nein	nein	nein	nein	nein	nein	nein
Signifikanz 99%								
Tabellenwert	1,944	1,944	1,944	1,944	1,944	1,944	1,944	1,944
Ausreißer?	nein	nein	nein	nein	nein	nein	nein	nein

Cochran-Test (Varianzenhomogenität) (Signifikanz 99%)		Mandel-F-Test auf Linearität (Signifikanz 99%)		Lineare Kalibrationsfunktion Y = a*x + b			
Prüfwert	0,345	Prüfwert	86,23	a	0,08506	R	0,9994
Tabellenwert	0,487	Tabellenwert	16,25	b	0,00382	Rest-SD	0,00595
Bestanden?	ja	Bestanden?	nein				

Prüfung Wichtungsfaktor			
Wichtung?	nicht erforderlich	Optimale Wichtung	---

Fig. 1. Data for instrument range and calibration range acquired with the ADH enzymatic method using the Thermo Scientific Indiko Analyzer.

3.2. Analytical Limiting Values

To determine the analytical limiting values (DIN 32645) we diluted the lowest calibrator (0.2 g/L) multiple times and measured these solutions in duplicate. For the ADH method using the Indiko Analyzer the Limit of Detection was 0.00 g/L and the Limit of Quantification 0.01 g/L (Fig. 2). The same results were obtained with the ADH method on the Technikon Autoanalyzer (Tab. 1).

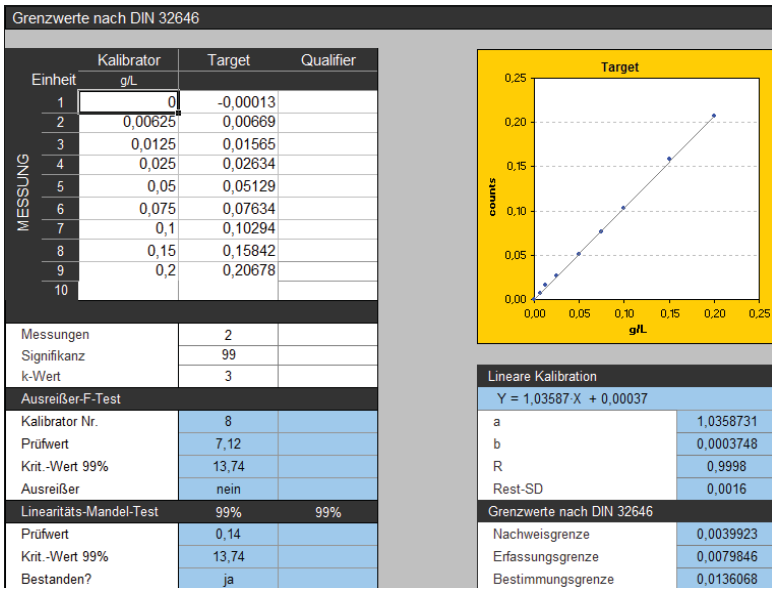

Fig. 2. Data for the analytical limiting values obtained with the Indiko Analyzer.

3.3. Accuracy

To determine the accuracy of the method we measured two quality controls (0.5 g/L and 2.0 g/L, Medichem) on 9 different days, each in duplicate at the beginning and at the end of a sequence. Analysis of these data is shown in figure 3. For the Indiko Analyzer, accuracy measurement with the serum control samples containing 0.50 or 2.00 g ethanol/L on 9 days yielded a mean result of 0.52 +/- 0.02 g/L and 1.98 +/- 0.06 g/L, respectively. The relative standard deviation (RSD) was 3.1% (0.50 g/L) and 3.2% (2.00 g/L); bias was 3.3% and -1.2%, respectively. Repeatability was calculated with RSD = 0.00% for both controls. The intermediate precision was RSD = 2.1% (0.50 g/L) and RSD = 1.5% (2.00 g/L). These validation parameters were similar to those obtained for the Technikon Autoanalyzer (Tab. 1).

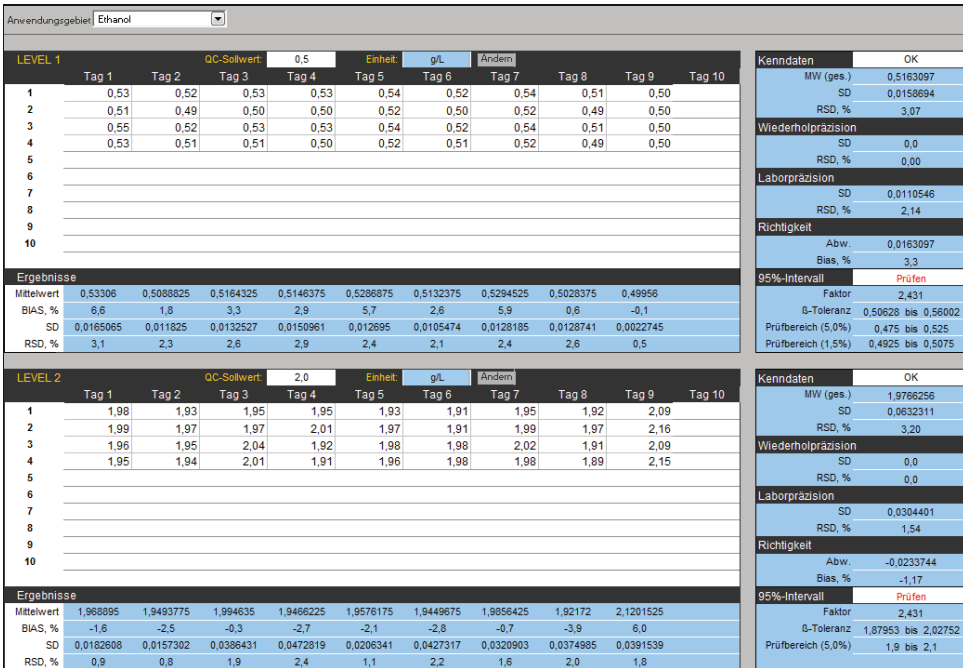

Fig. 3. Data for accuracy acquired with the Thermo Scientific Indiko Analyzer.

In summary, the validation parameters for the forensic BAC determination using the ADH enzymatic method on both devices provide similar results (Table 1). The results obtained for 93 authentic forensic serum specimens measured on both systems provide a linear regression equation of y=1.0202x-0.0087 and show an excellent correlation of r=0.9977 (Fig. 4).

Tab. 1. Summary and comparison of the validation data for the Thermo Scientific Indiko Analyzer and the Technikon Autoanalyzer.

	Thermo Scientific Indiko	**Technikon Autoanalyzer**
Mean recovery	100,5 %	100,5 %
Accuracy 0,5 g/L		
Mean	0,52 g/L, SD: 0,02, RSD: 3,1 %	0,50 g/L, SD: 0,01, RSD: 1,4 %
Accuracy of the mean	0,02 g/L	0,00 g/L
Bias	3,3%	0,9%
Repeatability	SD: 0,00, RSD: 0,0%	SD: 0,01, RSD: 1,6%
Intermediate precision	SD: 0,01, RSD: 2,1%	SD: 0,01, RSD: 1,6%
Accuracy 2,0 g/L		
Mean	1,98 g/L, SD: 0,06, RSD: 3,2%	2,03 g/L, SD: 0,03, RSD: 1,6%
Accuracy of the mean	-0,02 g/L	0,03 g/L
Bias	-1,2%	1,7%
Repeatability	SD: 0,00, RSD: 0,0%	SD: 0,01, RSD: 0,7%
Intermediate precision	SD: 0,03, RSD: 1,5%	SD: 0,03, RSD: 1,7%
Limiting values (DIN 32645)		
Limit of Detection	0,00 g/L	0,00 g/L
Limit of Decision	0,01 g/L	0,01 g/L
Limit of Quantification	0,01 g/L	0,01 g/L

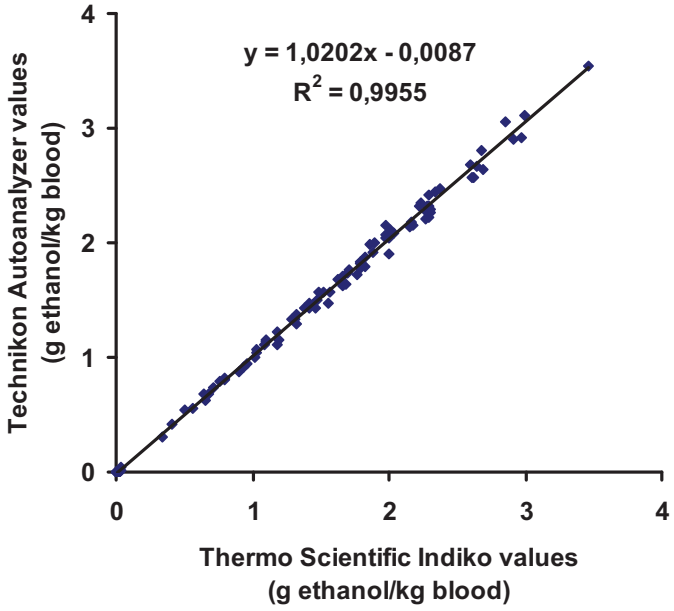

Fig. 4. Correlation data obtained from the BAC determination of 93 authentic forensic serum specimens measured on both devices.

4. Conclusions

Precision and reproducibility of the ADH method on the Thermo Scientific Indiko Analyzer meet the requirements for BAC determination in forensic specimens and were comparable to our currently used ADH method using the Technikon Autoanalyzer.

5. References

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