Fully automated determination of fluoride in blood samples

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Titration

Introduction

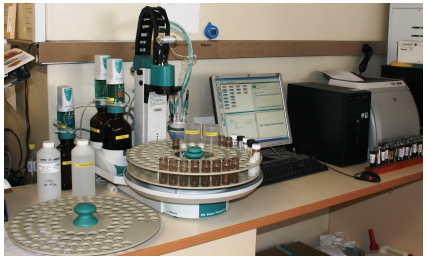
Sodium fluoride is used as a preservative in biological samples for alcohol analysis. All submitted blood samples, including those taken from vehicle drivers suspected of driving under the influence of liquor, have to be tested for adequate preservation prior to alcohol determination by gas chromatography. This is critical to ensure adequate sample preservation. Inadequate sample preservation may allow glycolysis and/or microorganism growth to produce ethanol.

In the past this has been done by direct potentiometric measurement using a fluoride-selective electrode (F ISE), an ion meter and certified NaF standards. The sodium fluoride level was determined manually by dipping the electrode directly into the blood sample. Results were recorded manually. This poster describes two independent automated methods of analysis that allow to minimize this tedious and time-consuming procedure.

In the first one, the fluoride content in a blood aliquot is measured by direct potentiometric measurement after the addition of TISAB and deionized water. The second method employs the titration of the sample aliquot with $La(NO_3)_3$ after adding a buffer solution.

Instrumentation

The system used for the automatic determination of fluoride in blood consists of a **855 Robotic Titrosampler** with three **800 Dosinos** for pipetting the blood samples and/or the NaF standards, adding TISAB or buffer solutions, titration with $La(NO_3)_3$ and a PC. The ***tiamo***™ software package controls the system. A right-side-fitted, micro-titration vessel lid is equipped with an F ISE and reference electrode, magnetic stirrer and PFA titration vessel. It also carries dosing tips, rinsing nozzles and an aspiration tip for aspirating spent sample solution to waste. Rinsing and waste containers are fitted with 849 Level Control for continuous monitoring of rinsing and waste levels to prevent pumps from running dry and/or waste containers from overflowing. Two different, specially manufactured custom-built sample racks are used for the fluoride analysis. Each of three different laboratories uses two automated systems with two different racks. The first one is for glass vials (322 places) while the second one is for special McCartney bottles (107 places). Two additional cap holding racks, specially developed for each of the two systems, store the caps during analysis as the samples are taken from either the opened glass vials or McCartney bottles. The cap holding racks were required since each vial, once opened, must be closed with the original cap after analysis for ensuring sample integrity.



Analytical procedure

Glass sample vials are used for samples taken from alleged drunken drive offenders and McCartney sample bottles are used for post-mortem blood samples from various mortuaries for alcohol-related fatal accidents and for insurance purposes. Incoming sample batches are logged on a LIMS and the sample data wirelessly imported into ***tiamo***™ via sample tables. The blood samples to be investigated are placed in the designated sample rack according to the relevant sample table. The subsequent sample transfer, direct potentiometric measurement or titration, calculation of results as well as the rinsing and cleaning steps take place fully automatically. Results are verified manually before exporting them wirelessly to the LIMS.

Pipetting of standards and blood samples

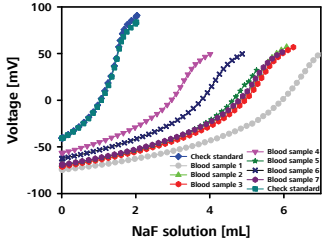
First, the transfer tube is completely filled with buffer solution (pH = 6). Subsequently, a fixed sample volume (1.5 mL aliquot for standards and samples alike), embedded between two air gaps, is aspirated from the sample vessel. Afterwards, the sample is transferred into the external titration vessel using a pipetting equipment that consists of the 800 Dosino with Dosing Unit (10 mL) and a 10-mL transfer tube with pipette tip. Finally the transfer tube is rinsed with 10 mL buffer solution into the titration vessel and 10 mL ultrapure water added.

Calibration

Three certified NaF standards (0.8%, 1.5%, 3.0%) are filled into PP vials and placed into three specially defined positions on the sample rack. The corresponding true values are entered as common variables STD 1…3. The sample series is started by recording a three-point calibration curve with certified NaF standards (beginning with 0.8%). The potential is measured under constant stirring and the mV value recorded. Thorough cleaning of the sensors, magnetic stirring bar, tubing, buret tips and the vessel is then carried out. This procedure is repeated with standards 2 and 3 (1.5% and 3.0% NaF, respectively). A three-point calibration curve is automatically recorded. The accuracy of the determination is verified after every 10 samples with a certified check standard (NaF content = 1.0%). The same sequence is followed for the determination of the fluoride content in blood samples.

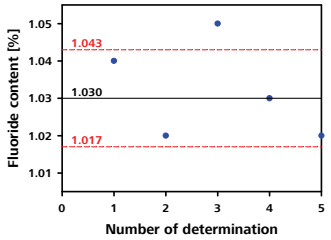
With the specially designed sample rack for glass vials the automated system can analyze 317 samples in one continuous run; the time required for each blood sample is 3 minutes including sample transfer and rinsing. In a similar way, the special rack for McCartney bottles allows to analyze 102 samples in one run.

Sample	Fluoride content [%]
Check standard (1.0% fluoride)	1.01
Blood sample 1	3.61
Blood sample 2	2.85
Blood sample 3	3.30
Blood sample 4	2.27
Blood sample 5	3.74
Blood sample 6	3.68
Blood sample 7	4.51
Check standard (1.0% fluoride)	1.02
Check standard (0.8% fluoride)	0.79
Check standard (1.5% fluoride)	1.53
Check standard (3.0% fluoride)	3.03



Fully automated titration

First, the transfer tube is completely filled with buffer solution (pH = 6). An air gap is created and a fixed volume (1.5 mL aliquot) of the sample is aspirated from the sample vessel followed by another air gap. The sample is transferred into the external titration vessel using pipetting equipment consisting of an 800 Dosino with Dosing Unit (10 mL) and a 10-mL transfer tube with pipette tip. The transfer tube is rinsed with 10 mL buffer solution into the titration vessel and 10 mL de-ionized water added. The sample is titrated with $c(La(NO_3)_3 = 0.1$ mol/L. Endpoint evaluation is done by using a Gran Plot. The titration vessel is rinsed and emptied several times, after which the system is ready for the next analysis. The same sequence is followed for the titer determination of the $c(La(NO_3)_3 = 0.1$ mol/L, which is carried out using a certified standard (3.0% NaF).



Check standard (1% fluoride)	Fluoride content [%]
Determination 1	1.04
Determination 2	1.02
Determination 3	1.05
Determination 4	1.03
Determination 5	1.02
Mean	1.03
RSD	1.3%

Summary

Drunk driving causes many accidents all over the world. Therefore, most countries forbid operation of motor vehicles above prescribed levels of blood alcohol content (BAC). BAC can be objectively measured and defines intoxication. However, under the action of enzymes and bacteria the blood sugar can be fermented into ethanol, which increases the BAC. The addition of a known amount of fluoride, which acts as an enzyme inhibitor, suppresses alcohol formation. Therefore, in some countries not only the BAC value but also the fluoride content of the sample has to be reported.

It has been shown that the fully automated determination of fluoride in blood samples can be carried out by precipitation titration with $La(NO_3)_3$ or, at lower concentration, with the standard addition method. Thanks to constant pipetting and measurement procedures, the presented system offers more accurate results.

References

(1) Metrohm Application Bulletin AB-082, Determination of fluoride with the ion-selective electrode, http://products.metrohm.com (search for AB-082).

Acknowledgements

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Download a copy of this poster from http://products.metrohm.com (search for 8.000.6048EN).

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