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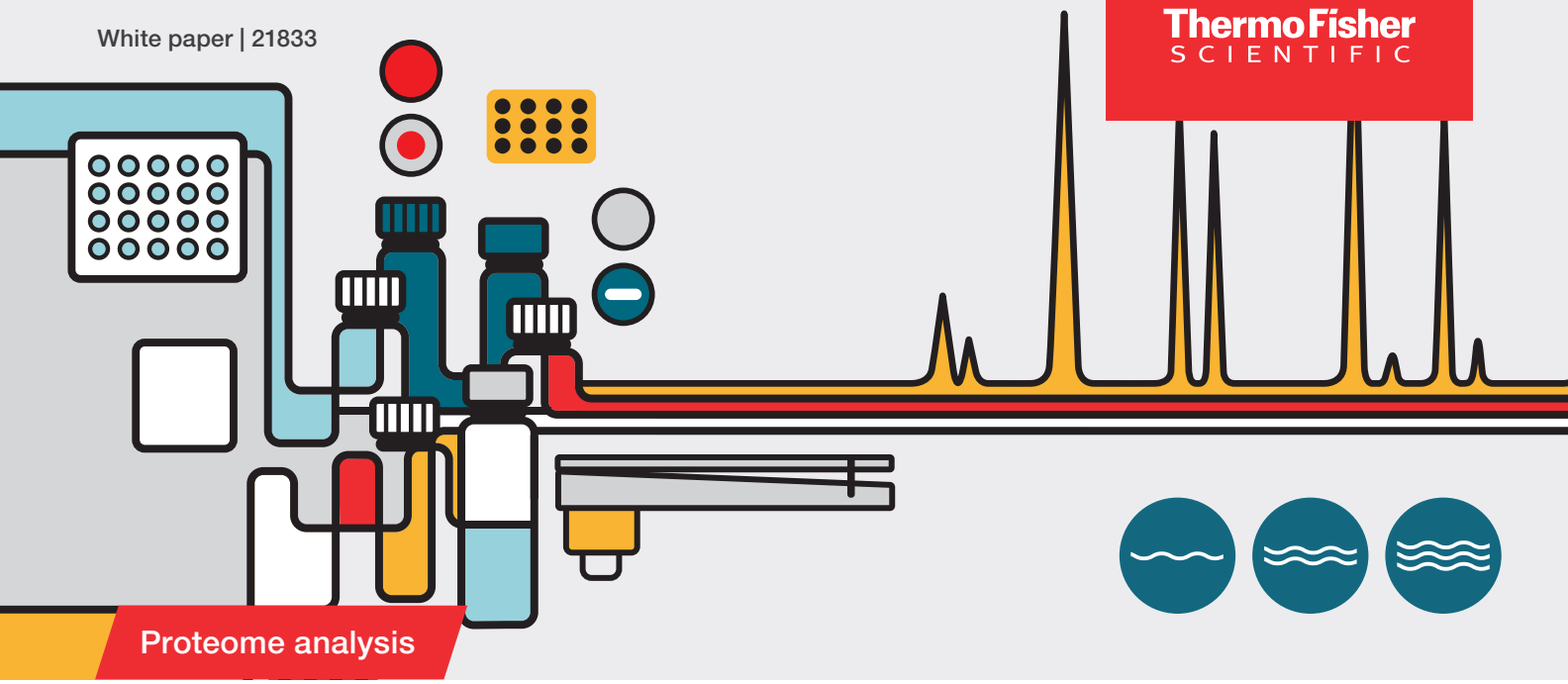

Proteome analysis

Why is GOLD-grade glass quality so important when you want to detect low concentrations of analytes?

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Keywords

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Abstract

Have you ever thought about the quality of your glass vial? If not, this study will give you an overview, why glass quality is so important, especially when you want to detect small concentrations or certain structures of analytes.

In this study drugs (e.g. Doxepin, Clomipramin, Bromperidol (containing trisubstituted N-atoms = tertiary amines and other TCA´s) have been investigated in serum in order to see the effects of certain glass qualities. The results are reported by reproducibility data using different types of 2 mL autosampler glass vials.

Introduction

When we talk about glass quality, we mostly talk about first hydrolytic class borosilicate glass with different expansion coefficients, which range from 70 type basic glass to 51 type, up to 33 type. What does that mean? Expansion coefficient actually describes the activity of the surface of the glass wall, which relates to the amount of free silanol groups present that can react with analytes and bind them to the glass surface.

70 type glass can be seen as having a surface with about 70% free silanol groups. Additionally, as the following pictures show, the glass surface is not even. Coupled with large number of free silanol groups, these surface imperfections (irregular surface structure as well as scratches and holes) lead to higher surface activity. Higher surface activity increases the amount that a susceptible analyte will stick to, or be adsorbed,

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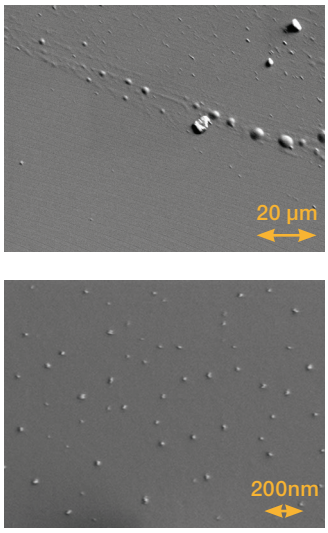
on the surface. This problem is further compounded because the degree of adsorption varies over time, particularly when complex matrices are present (for example in the presence of serum). When analysing low concentration of susceptible analytes the percentage of analyte adsorbed is relatively greater and the scale of the problem is compounded. This results in less free analyte available for analysis on the chromatographic system and potentially incorrect results.

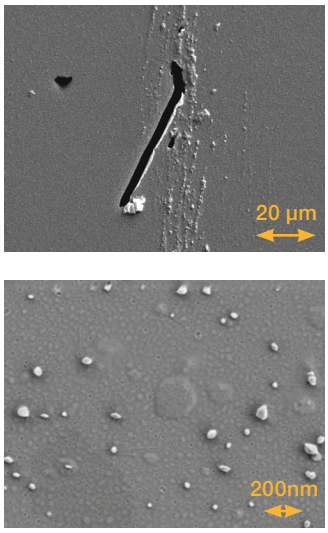
Thermo Scientific vials and closures

Vial/Glass quality comparison, 1st hydrolytic class glass types, 33 vs 70.

33 expansion glass, clear



70 expansion glass, clear



The determination of TCA´s (Tricyclic antidepressants) out of a biological matrix like serum or plasma is a daily routine analysis for laboratories dealing with drugs and serum levels. In a real matrix, some TCA´s tend to adsorb strongly on the walls of the sample handling container, like a glass vial. This effect is time sensitive, as with longer time frames in between sample preparation and LC/MS analysis, the quantity of accessible drug varies significantly. If the sample sits too long on the rack, this effect is bigger. In reality, those samples have to be measured immediately to receive results that are not influenced by the surface of the glass. The routine in a lab may have to be interrupted, to make sure, that the critical samples are measured first.

This interruption of the routine workflow costs a lot of time and increases the risk of result failure for the sequences. Additionally the level of stress is significantly increased as these “in-between” actions need to be documented and included in SOP´s, etc.

In this study drugs (e.g. Doxepin, Clomipramin, Bromperidol (containing tertiary amine) and other TCA´s) have been investigated in real matrix (serum) in order to get reproducibility data using different types of 2 mL autosampler glass vials.

The target was to find a vial type with a non-adsorbing surface that allows the TCA sample to be included in the normal workflow without interrupting the currently running sequences. For this, the requirement is no interaction in between the analytes and the glass surface, including an excellent vial-to-vial reproducibility.

The vials used for the experimental part

All the vials used are from 1st hydrolytic class borosilicate glass, but with different expansion coefficients, reaching from 33 type to 51 type basic glass. One vial was additionally silanized, meaning that the surface is inactivated.

Vial A	clear glass	33 type	made in Germany	6PSV9-1PG
Vial B	clear glass	51 type	made in Germany	6ASV9-1P
Vial C	clear glass	51 type	made in Asia	C
Vial D	clear glass	51 type	made in Europe	D
Vial E	clear glass silanized	51 type	made in Europe	E

Instrumentation/method

Time experiment from 0 hrs. to 24 hrs. (sample on rack)

UHPLC System:	Thermo Scientific™ UltiMate™ 3000 UHPLC System
Detector:	Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS System
Column:	Thermo Scientific™ Hypersil GOLD™ C18 Selectivity LC Column (100 × 2.1 mm (P/N 25003-102130)
Sample:	Humanalbumin (ALBUNORM) is spiked with a target standard of 150 ng/mL for each of the TCA`s of interest. The 150 ng/mL standard reflects a middle-range quantitation standard.

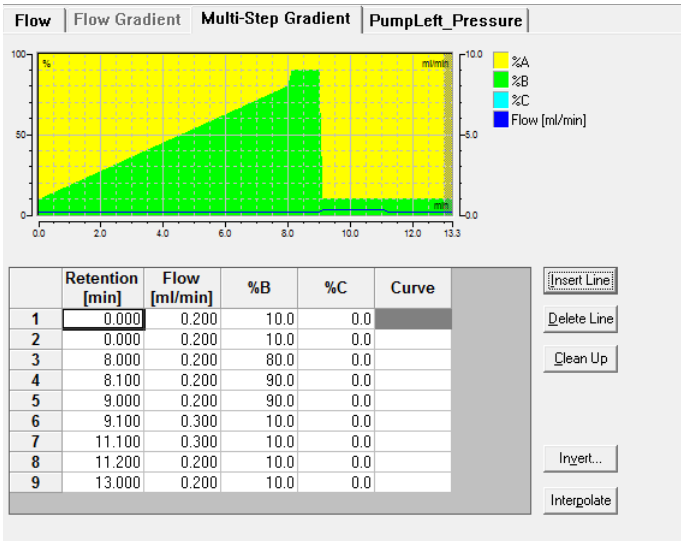
Following Rili-BAEK regulations (Richtlinie der Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen) the acceptance criteria for accuracy is 25% (112.5 – 187.5 ng/mL).

Reagents used

- Multi Internal Standard (40 ng/mL Bromperidol in ACN LCMS Grade)
- Orbitrap Buffer (1000 mL Water LCMS, 0.05 % formic acid, 100 mL MeOH LCMS)
- External control (suitable external control standard)
- TCA calibration (TCA K1 – K3)
- TCA Precision

Protocol

- 100 µL of standard, or sample, are pipetted into a polypropylene centrifuge tube.
- 400 µL of multi internal standard is added.
- Set immediately on “lab dancer” and centrifuge afterwards.
- 400 µL of Orbitrap buffer is pipetted into a glass vial.
- 400 µL of sample, or standard, is added to the glass vial and shaken.
- Inject on the Q Exactive Orbitrap (injection volume 5 µL)
- LC Method Used the Thermo Scientific UltiMate 3000 LC System with a multistep gradient

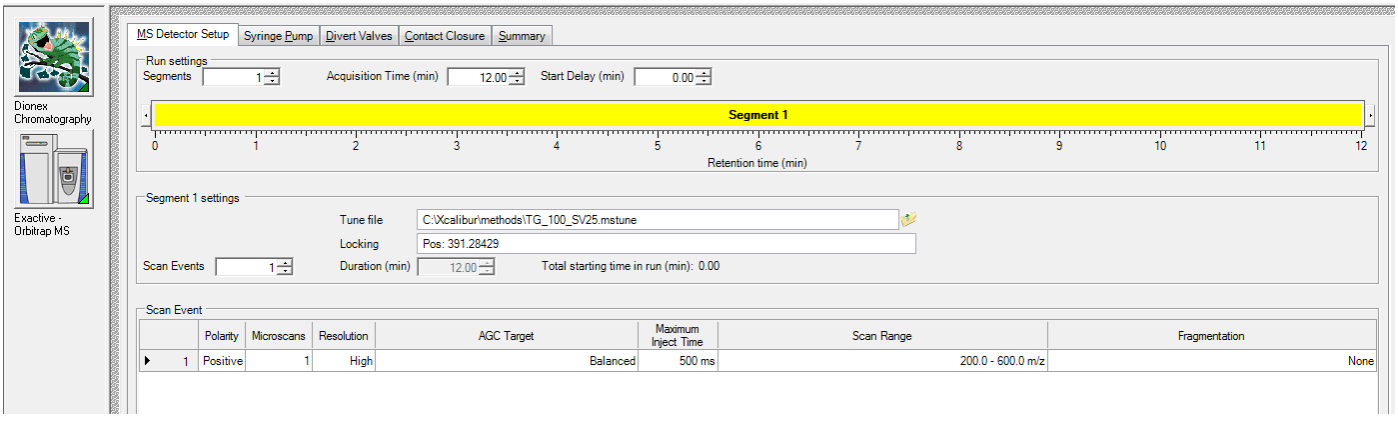


	Retention [min]	Flow [ml/min]	%B	%C	Curve
1	0.000	0.200	10.0	0.0	
2	0.000	0.200	10.0	0.0	
3	8.000	0.200	80.0	0.0	
4	8.100	0.200	90.0	0.0	
5	9.000	0.200	90.0	0.0	
6	9.100	0.300	10.0	0.0	
7	11.100	0.300	10.0	0.0	
8	11.200	0.200	10.0	0.0	
9	13.000	0.200	10.0	0.0	

Oven temperature 40° C

	Quan Peak	Compound Name	Trace
1			TIC
2			A/D1:Ch1
3			A/D1:Ch2
4			UV1:ColumnOven_Temp
5			UV2:PumpLeft_Pressure
6	•	Doxepin	m/z280.16959
7	•	Fluperlapin	m/z310.17140
8	•	Nordoxepin	m/z266.15394
9	•	Amitriptylin	m/z278.19033
10	•	Bromperidol	m/z420.09690
11	•	Desimipramin	m/z267.18558
12	•	Imipramin	m/z281.20123
13	•	Trimipramin	m/z295.21688
14	•	Clomipramin	m/z315.16225
15	•	Nortriptylin	m/z264.17468

Accurate Mass Traces Used For TCA`s



Thermo Scientific Q Exactive Orbitrap MS Parameters

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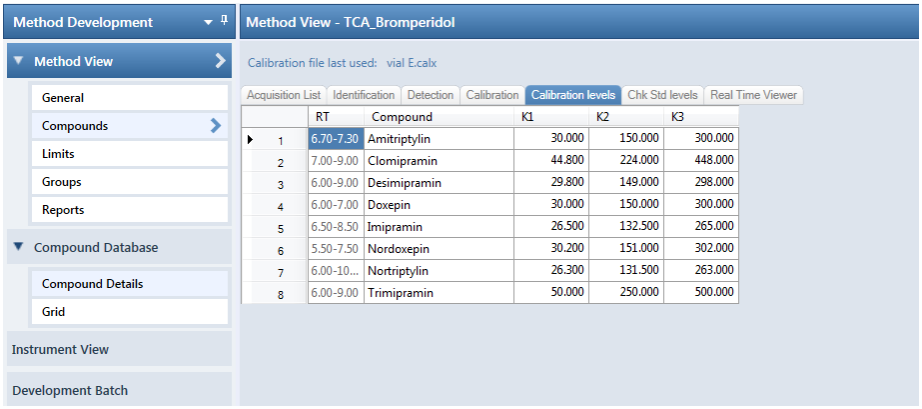
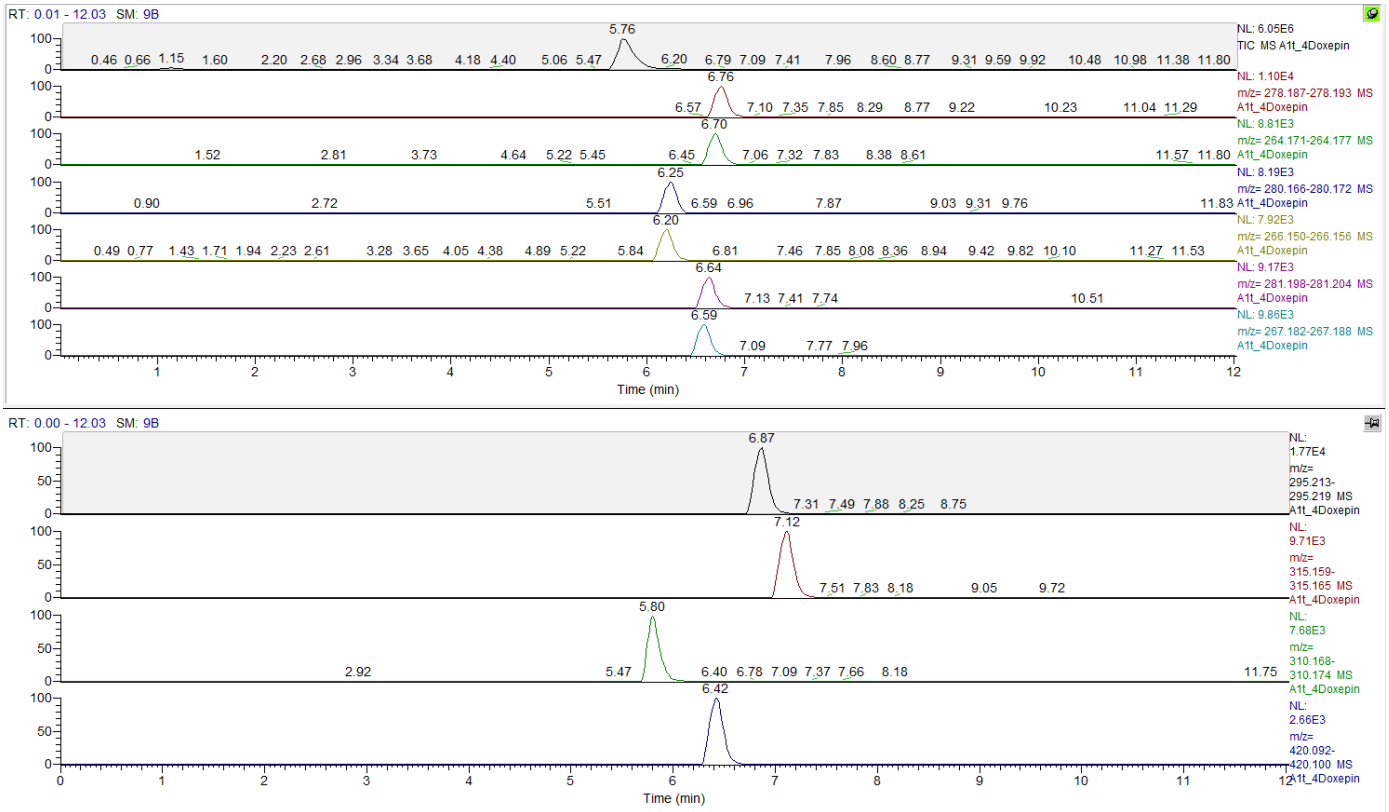


	RT	Compound	K1	K2	K3
1	6.70-7.30	Amitriptylin	30.000	150.000	300.000
2	7.00-9.00	Clomipramin	44.800	224.000	448.000
3	6.00-9.00	Desimipramin	29.800	149.000	298.000
4	6.00-7.00	Doxepin	30.000	150.000	300.000
5	6.50-8.50	Imipramin	26.500	132.500	265.000
6	5.50-7.50	Nordoxepin	30.200	151.000	302.000
7	6.00-10...	Nortriptylin	26.300	131.500	263.000
8	6.00-9.00	Trimipramin	50.000	250.000	500.000

Quantification Method for TCA`s

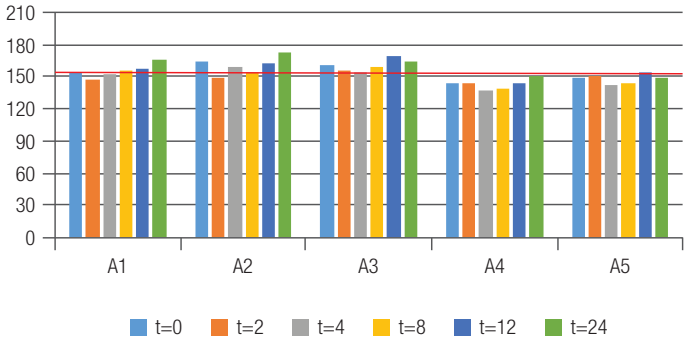


Overview *m/z* for TCA`s and IS in Excalibur

Results

In general all data reflect five different vials from one lot. Results taken directly after filling the vial and injection took place after 0, 2, 4, 12 and 24 hours.

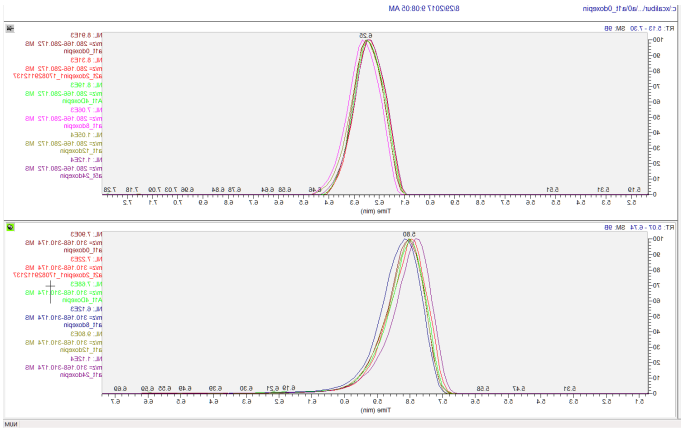
Overview vial A (Thermo Scientific™ SureSTART™ GOLD-grade glass Vial; P/N: 6PSV9-1PG)



This graph shows a GOLD-grade glass vial

The picture shows excellent recovery and reproducibility from vial-to-vial and over a long period of 24 hours. The values differ only 13% of the target value for 25 runs out of five different vials, over 24 hours.

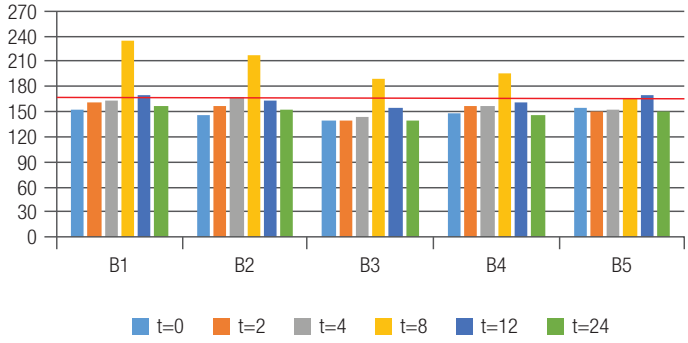
Areas of Doxepin and Bromperidol (IS) Overlay Mode



This chromatogram shows an overlay for Doxepin

The overlay shows perfect results for Doxepin compared to the internal standard Bromperidol. Peaks show the same heights and symmetry and a very good recovery and reproducibility.

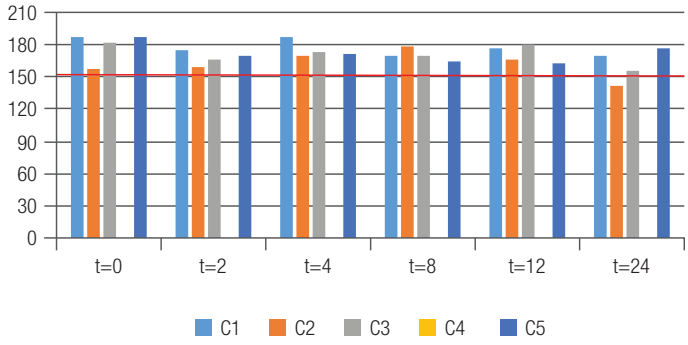
Overview vial B (SureSTART GOLD-grade glass Vial; P/N 6ASV9-1P)



This graph shows a 51 type glass made in Germany

The reproducibility for the 51 type glass vial is not as good as the reproducbility for the SureSTART™ GOLD-grade glass vial, (the variance is 35% at the maximum). The reason for this is the 51 type glass quality (from Germany). As discussed above, the surface is not as even as using the GOLD-grade glass vial. This figure shows the effect of the free silanol groups interacting with the Doxepine containing trisubstituted N-atoms. Analytes containing trisubstituted N-atoms are more likely to be affected by the free silanol groups on the glass surface. Therefore it is very important to use a good glass quality. Also if low detection limits need to be reached, the surface has a high impact on the results. Using a 51 or 70 type glass can result in loss of low calibration points in a calibration curve.

Overview vial C

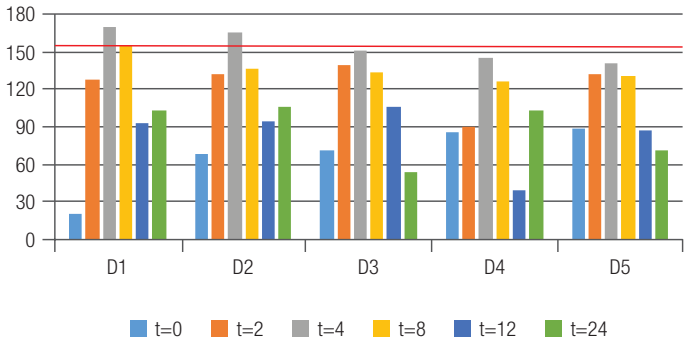


The data reflect a 51 type glass (from Asia)

In this case four vials were tested out of one lot. Again, the analysis was performed after 0, 2, 4, 12 and 24 hours.

Three vials always showed a way-too-high recovery; one vial showed in a non-reproducible way, too-high and too-low results. The variance was up to 34%. The reproducibility is acceptable, but not good.

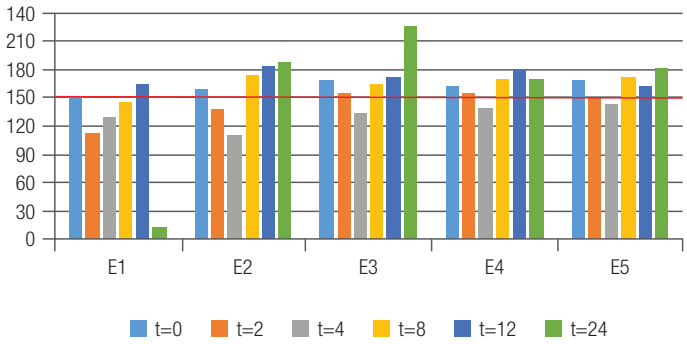
Overview vial D



These results reflect another 51 type glass (from Europe)

As the graph shows, the results are not acceptable. Only two of the five vials show a kind of constancy. The others show a variance of about 86%, which is not acceptable at all. In short, all vials show a decrease of the Doxepin concentration over time.

Overview vial E



This graph shows a silanized, 51 type glass (from Europe)

The silanized vial was expected to be excellent, because of the covered surface; but the real data shows a sometimes significant variance from vial-to-vial. The reason for this significant variance is a disturbance on the exact *m/z* that is generated from the silanization reagent. The maximum variance for this glass type is 86%. The total recovery over time is more or less constant compared to the 51 type vial D (from Europe).

Conclusions

- For this study it was important that real serum was tested using internal standards for quantification, as no routine laboratory will run TCA`s in pure water. The most drastic adsorption effect on different types of glass was monitored with the compound Doxepin due to the fact, that it contains trisubstituted N-atoms.
- Other substances that contain trisubstituted N-atoms from the class of the TCA`s are not so sensitive here and show more uniform results from vial-to-vial and over time. The Thermo Scientific SureSTART™ GOLD-grade glass vials showed the best results for all TCA`s including the most critical, Doxepin. This effect was shown over five different vials for a period of up to 24 hours. The variance of only 13% support the superior chemistry and performance of the SureSTART™ GOLD-grade glass vials surface. These vials, which were introduced to the market in 1989 for critical compounds, are still a leading product in terms of ultra low adsorption for critical compounds.
- This article shows how important the quality of glass is in order to achieve good analytical results. As demonstrated, a 51 type glass can vary as well, depending on the country of origin.
- If low concentration levels need to be measured, the glass vial is more important than the instrument used. If the analyte sticks on the glass wall, it is not injected into the chromatographic system. The instrument measures only what is injected. The key for success reaching low levels is the glass vial.

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