Influence of Glass Vial Type Upon Trace Level Recovery Rates of Basic Analytes by LC/MS/MS

Matthias Kamuf[1] & Adam G Harder[2]; [1]Agilent Technologies GmbH, Waldbronn, Germany, [2]Agilent Technologies, Englewood CO, USA

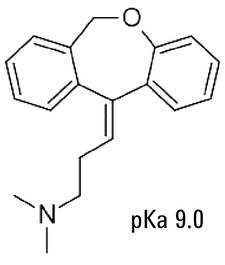
Agilent Technologies

Introduction

This investigation highlights sample vial selection as an essential consideration when performing quantitative analyses of basic molecules at low detectable levels. With ongoing advances in chromatography and mass spectrometry instrumentation pushing detection limits ever lower, even trace analyte loss due to interaction with the sample vessel can impact the quality and consistency of results. This is of particular importance when analyzing long automated sample sequences where reliability of results over time is essential.

Premium glass vials manufactured from Type 1 borosilicate glass contain a wide array of metals at the glass surface (e.g. Na, Mg, Al, Fe, Mn, etc...) which can vary between manufacturing processes, and sometimes even lot-to-lot (or batch-to-batch). These surface variations can effect the adsorption behavior of analytes stored within the vial, particularly with basic molecules, in ways that we cannot fully predict. Here we assess a range of different premium (low adsorption) glass autosampler vial types focusing on differences in recovery rates of doxepin as monitored using LC/MS/MS.

Doxepin Chemical Structure



The tricyclic antidepressant Doxepin was chosen as a model compound to assess the interaction of basic analytes with the interior surface of glass vials and/or with impurities in the vessels' material.

Methods

LC Parameters

Parameter	Value
LC System	Agilent 1290 Infinity UHPLC
Column	Agilent InfinityLab Poroshell 120 EC-C18 2.1 x 50 mm; 2.7 µm
Column temperature	50 °C
Sample Temperature	20 °C
Mobile phase A	H_2O; 0.1% formic acid
Mobile phase B	Acetonitrile; 0.1% formic acid
Eluent	60% A, 40%; isocratic
Flow rate	0.6 mL/min
Detection	MS-QQQ
Sample	1 mL of 1 ppb (m/V) Doxepin (as Hydrochloride) in Eluent
Injection Volume	1.0 µL

MS Parameters

Parameter	Value
MS System	Agilent G6495 QQQ w/ AJST source
Ion Mode	Positive
Drying gas temperature	275 °C
Drying gas flow	12 L/min
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Nebulizer pressure	35 psi
Capillary voltage	4,000 V
Nozzle voltage	1,500 V
Scan type	SRM : 280.1 => 107.0 m/z
MS1 & MS2 resolution	Wide

Experimental Approach

The amount of sample loss due to adsorption within each vial type was assessed by looking at doxepin recoveries over time. Recoveries less than 100% can be attributed to adsorption of doxepin to the interior surface of the test vial, as nothing changes within the system except the test vial itself. Six vials from each lot were assessed for statistical significance and test vials were randomized within each sequence. Repeat injections from the same glass test vial were performed in four hour, or more, increments (i.e. *t0, t4, t8, t12, t16, t20, t24, t36, t48*).

Note: Tests were carried out by Agilent. Both clear and amber formats were tested for Agilent A-Line vials; all other competitor vials tested were clear format only.

Calculations

Recovery

Recoveries of doxepin from each vial at '*x*' time-point(s) (%Recovery$_{tx}$) were calculated by comparing the SRM peak areas from the glass test vials (*vial*$_{tx}$) to the peak areas of the same doxepin stock solution stored in polypropylene vials at time zero (*PP*$_{t0}$).

$$\%\text{Recovery}_{tx} = \frac{\text{Area}(vial_{tx})}{\text{Area}(PP_{t0})} \times 100\%$$

Note: Meticulous care was taken to create a true time zero (*t0*) starting point for each vial by adding aliquots of the same 1 ppb doxepin stock solution into each vial immediately preceding injection via the autosampler. (*t0* = 30sec ± 10sec)

Precision

The precision of the recovery measurements were determined by calculating the relative standard deviations (RSDs) for both vial-to-vial as well as lot-to-lot.

$$\%\text{RSD}_{vial-to-vial} = \frac{SD_{tx}}{V_{tx}} \times 100\%$$

SD_{tx} = Standard deviation of the %Recovery$_{tx}$ values for all 6 vials
V_{tx} = Average %Recovery$_{tx}$ of all 6 vials within a lot (n=6)

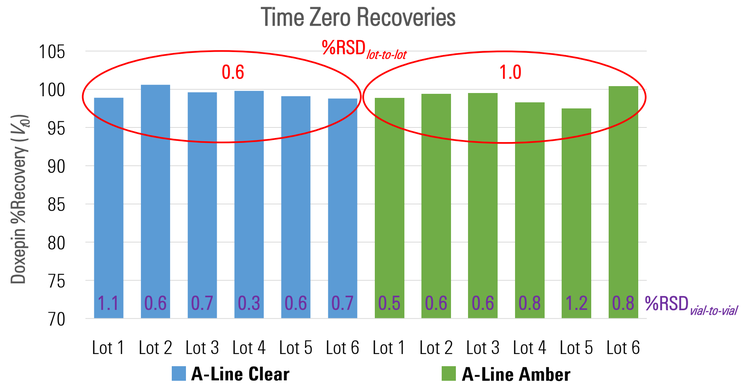
$$\%\text{RSD}_{lot-to-lot} = \frac{SD(V_{tx})}{L_n(V_{tx})} \times 100\%$$

$SD(V_{tx})$ = Standard deviation of the V_{tx} values for all 'n' lots
$L_n(V_{tx})$ = Average V_{tx} of all 'n' lots

Results and Discussion

Agilent A-Line Vials

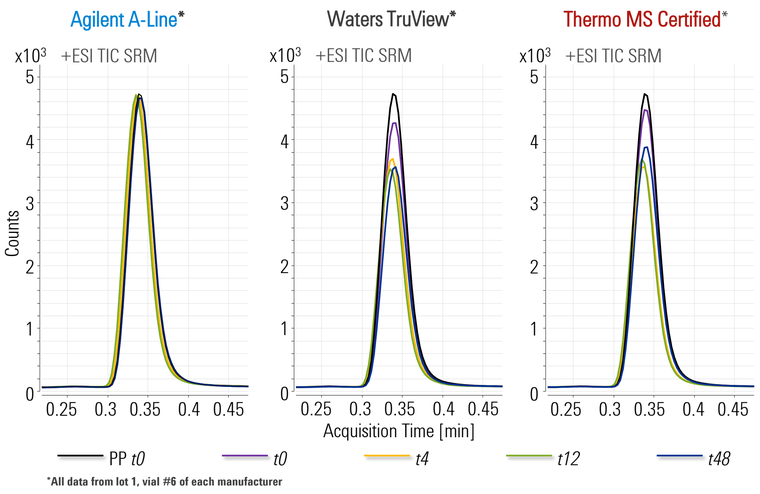
Consistency of the A-Line vial manufacturing process produces increased precision of response, both from one vial to another within each lot (vial-to-vial), as well as from one lot to another (lot-to-lot).



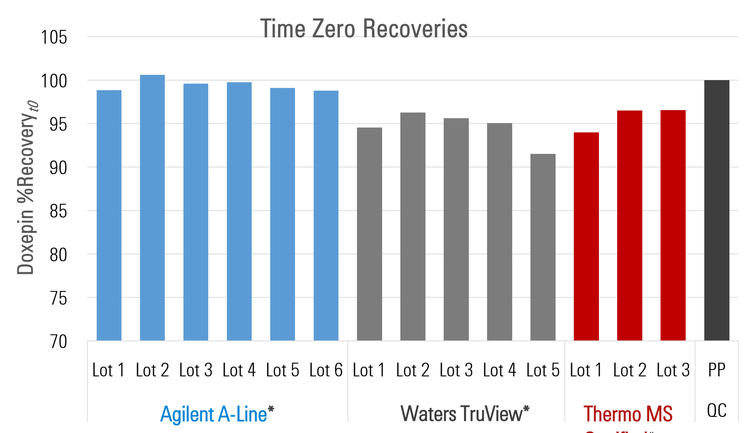
A-Line vials show consistency of response within each lot as can be seen by the low vial-to-vial RSDs. Lot-to-lot precision is also excellent for both A-Line clear and amber vials, with observed lot-to-lot RSDs of 0.6% and 1.0% respectively.

Analyte Loss Over Time

Visible differences in chromatographic peak areas can be observed between different vial types over time.

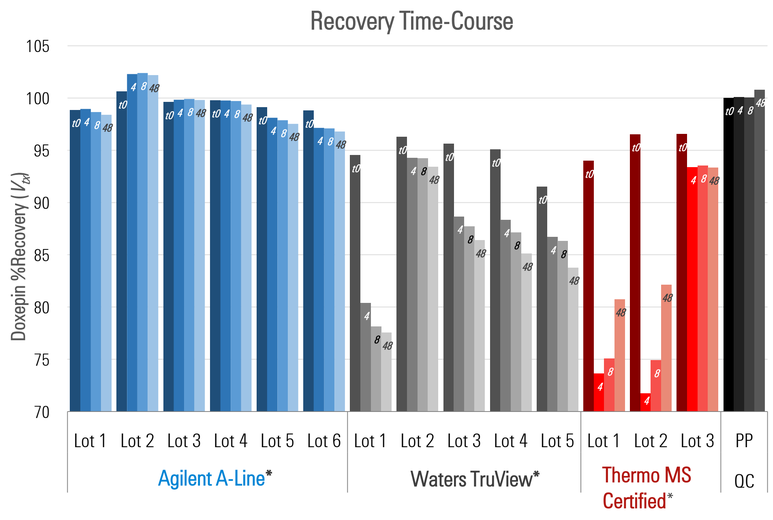


Some minor, but rapid losses were observed directly after adding the sample into the vials at *t0* (30sec ± 10sec).



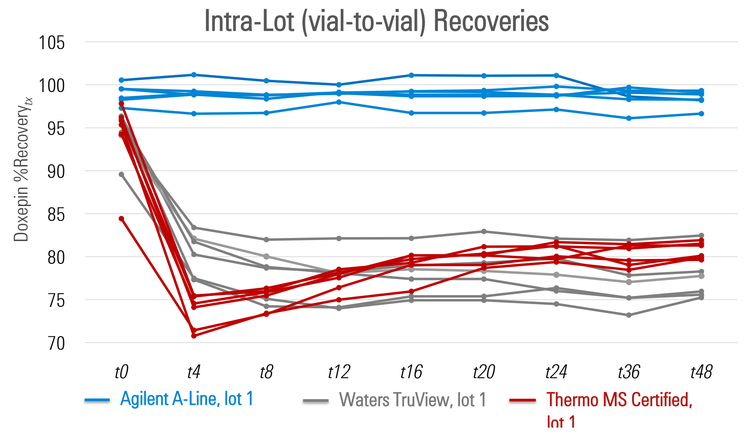
Results and Discussion

The majority of detectable losses occurred within the four hour time-point, with primarily minor changes observed for the later time points, suggesting the adsorptive loss due to interaction with the vial surface is a relatively fast process.



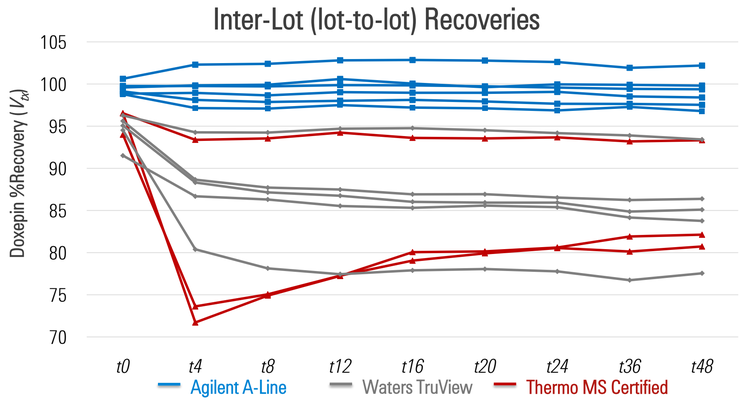
Vial-to-Vial Comparison

The chart below illustrates an example of the variability within individual lots by plotting the %Recovery$_{tx}$ of doxepin from individual vials within a single lot over time. (Note: each data point represents one vial.)



Lot-to-Lot Comparison

The chart below illustrates the average recovery rates of the A-Line vials compared to different competitor premium vial products. (Note: each data point is the average of n=6 vials.)



The table below summarizes the recovery and precision values for the entire time-course.

		% Recovery$_{tx}$					%RSD$_{vial-to-vial}$					% RSD$_{lot-to-lot}$				
		t0	*t4*	*t12*	*t24*	*t48*	*t0*	*t4*	*t12*	*t24*	*t48*	*t0*	*t4*	*t12*	*t24*	*t48*
Agilent A-Line	Lot 1	98.9	99.0	99.0	99.1	98.4	1.3	1.1	0.6	1.2	0.9					
	Lot 2	100.6	102.3	102.8	102.6	102.2	0.6	0.6	0.5	0.8	0.7					
	Lot 3	99.6	99.8	100.6	100.0	99.8	0.3	0.7	0.1	0.3	0.9	0.6	1.6	1.8	1.8	1.8
	Lot 4	99.8	99.8	99.9	99.6	99.4	0.5	0.3	0.5	0.5	0.7					
	Lot 5	99.1	98.1	98.0	97.7	97.5	1.2	0.6	1.0	0.9	0.6					
	Lot 6	98.8	97.1	97.5	96.9	96.8	0.6	0.7	0.9	0.8	0.7					
Waters TruView	Lot 1	94.5	80.4	77.4	77.8	77.5	2.9	2.4	3.6	3.3	3.2					
	Lot 2	96.3	94.3	94.7	94.2	93.4	1.5	1.8	1.5	1.4	1.1					
	Lot 3	95.6	88.7	87.5	86.5	86.4	1.7	2.3	2.0	2.0	2.8	1.7	5.1	6.4	6.1	6.0
	Lot 4	95.1	88.3	86.7	85.9	85.1	0.8	1.6	0.7	1.2	1.4					
	Lot 5	91.5	86.7	85.5	85.4	83.8	0.9	5.6	1.8	0.8	0.4					
Thermo MS Certified	Lot 1	94.0	73.6	77.3	80.5	80.7	2.5	4.7	1.6	1.1	1.1					
	Lot 2	96.5	71.7	77.2	80.6	82.1	0.9	1.8	0.9	1.2	1.5	1.3	12.3	9.7	7.3	6.6
	Lot 3	96.5	93.4	94.2	93.7	93.3	0.3	1.8	0.1	0.3	0.9					
QC	PP	100.0	100.1	100.6	100.3	100.8	0.4	0.6	0.1	0.7	1.0	-	-	-	-	-

% Recovery	≥ 95.0	95.0 - 90.0	≤ 90.0
% RSD	≤ 2.0	2.1- 3.0	≥ 3.0

Conclusions

- **Sample vial selection is an essential consideration when performing quantitative LC and/or LC/MS analyses of basic analyte at low detectable levels.**
- **Compared to other vendor's premium vial options, the Agilent A-Line vials demonstrated the overall lowest adsorptive loss of doxepin.**
- **The Agilent A-Line vials demonstrated excellent precision of measurement and consistency of response over time from vial-to-vial and lot-to-lot.**

Learn more: www.agilent.com/chem/vialsresources

Printed in US February 9, 2017
5991-7712EN