File: Supplementary materials

Supplemental Materials

Table S1. Spectrophotometric data set complete recorded on an Epoch microplate reader (Epoch, Biotek UK) for all test sample combinations of Chemfort™ SAL/ LL syringes at a detection wavelength of 660 nm.

Chemfort™ SAL/ LL 50mL test syringes			Chemfort™ SAL/ LL 20mL test syringes			Chemfort™ SAL/ LL 1mL test syringes		
Sample	**Average abs. at 660 nm**	**Pass/Fail**	**Sample**	**Average abs. at 660 nm**	**Pass/Fail**	**Sample**	**Average abs. at 660 nm**	**Pass/Fail**
Syringe 1	-0.004	Pass	Syringe 1	-0.002	Pass	Syringe 1	-0.005	Pass
Syringe 2	-0.003	Pass	Syringe 2	-0.004	Pass	Syringe 2	-0.004	Pass
Syringe 3	-0.003	Pass	Syringe 3	-0.004	Pass	Syringe 3	-0.002	Pass
Syringe 4	-0.003	Pass	Syringe 4	-0.003	Pass	Syringe 4	-0.004	Pass
Syringe 5	-0.003	Pass	Syringe 5	0.001	Pass	Syringe 5	-0.007	Pass
Syringe 6	-0.004	Pass	Syringe 6	-0.002	Pass	Syringe 6	-0.006	Pass
Syringe 7	-0.002	Pass	Syringe 7	-0.002	Pass	Syringe 7	-0.003	Pass
Syringe 8	-0.005	Pass	Syringe 8	-0.003	Pass	Syringe 8	-0.009	Pass
Syringe 9	-0.004	Pass	Syringe 9	-0.005	Pass	Syringe 9	-0.003	Pass
Syringe 10	-0.004	Pass	Syringe 10	-0.004	Pass	Syringe 10	-0.004	Pass
Syringe 11	-0.007	Pass	Syringe 11	-0.005	Pass	Syringe 11	-0.008	Pass
Syringe 12	-0.006	Pass	Syringe 12	-0.006	Pass	Syringe 12	-0.008	Pass
Syringe 13	-0.005	Pass	Syringe 13	-0.003	Pass	Syringe 13	-0.007	Pass
Syringe 14	-0.007	Pass	Syringe 14	-0.006	Pass	Syringe 14	-0.006	Pass
Syringe 15	-0.005	Pass	Syringe 15	-0.005	Pass	Syringe 15	-0.004	Pass
Syringe 16	-0.004	Pass	Syringe 16	-0.004	Pass	Syringe 16	-0.008	Pass
Syringe 17	-0.004	Pass	Syringe 17	-0.004	Pass	Syringe 17	-0.005	Pass
Syringe 18	-0.004	Pass	Syringe 18	-0.004	Pass	Syringe 18	0.006	Pass
Syringe 19	-0.009	Pass	Syringe 19	-0.010	Pass	Syringe 19	-0.010	Pass
Syringe 20	0.001	Pass	Syringe 20	-0.008	Pass	Syringe 20	-0.005	Pass
			Syringe 21	-0.006	Pass			

File: Supplementary materials

Table S2. Spectrophotometric data set complete recorded on an Epoch microplate reader (Epoch, Biotek UK) for all combination devices of sterile blind hub/ LL syringes as positive control samples with detection at wavelength of 660 nm.

Sterile blind hub/ LL 50mL positive control syringe combinations		Sterile blind hub/ LL 20mL positive control syringe combinations		Sterile blind hub/ LL 1mL positive control syringe combination	
Sample	**Average absorbance at 660 nm**	**Sample**	**Average absorbance at 660 nm**	**Sample**	**Average absorbance at 660 nm**
P1	4.032	P1	1.115	P1	0.048
P2	3.655	P2	4.101		
P3	3.895	P3	0.615		
P4	3.329				
P5	1.850				

Validation of the Spectrophotometric method:

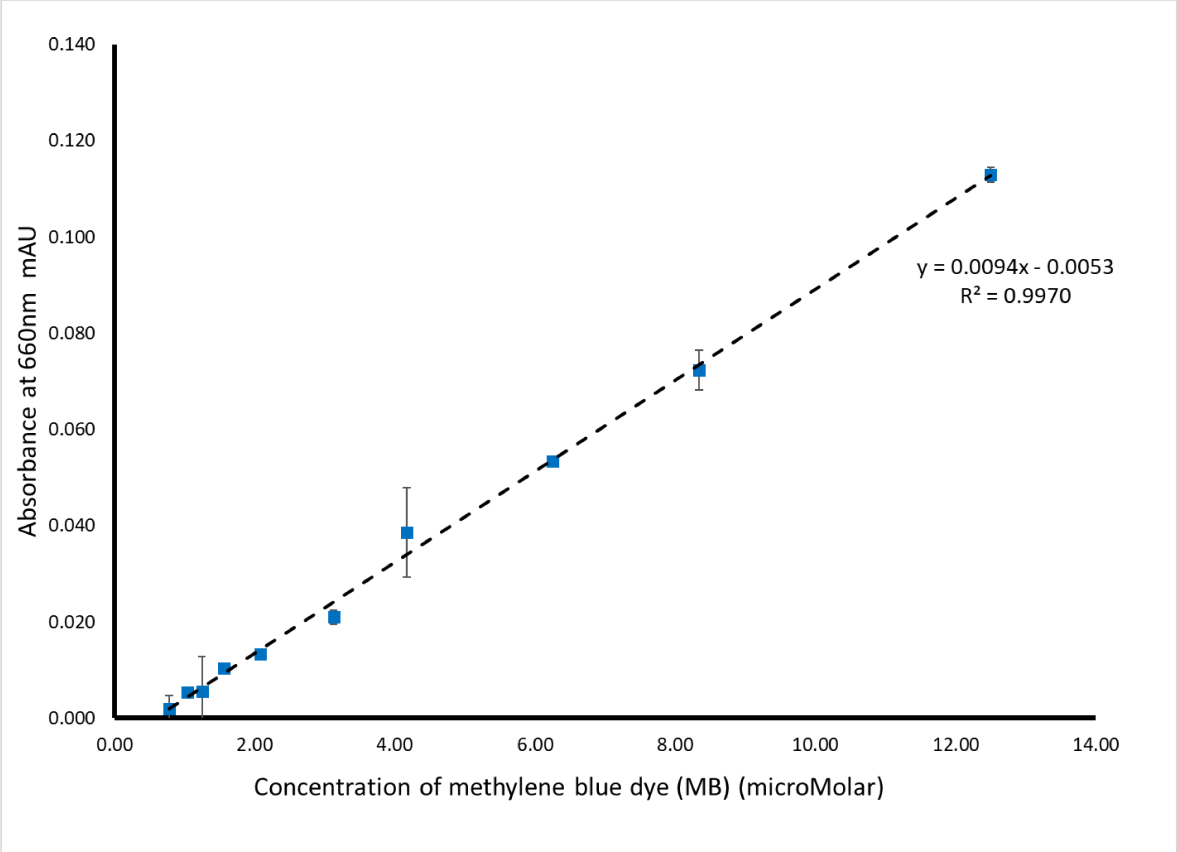
The methylene blue dye intrusion test method was validated as a limit test according to ICH guidelines. That is to say it results in either a pass or a fail outcome. The limit of detection (LOD) for dye penetration should according to ICH guidelines correspond to the smallest level of dye added to a product that is still consistently detectable. Validation of the spectrophotometric method was performed in accordance with NHS PQA guidance for Pharmaceutical Quality Control Analytical Methods and ICH Q2 (R1) guidance for validation of analytical methods.[1,2]

For specificity testing in accordance with ICH guidelines the dye intrusion method was assessed as to whether it demonstrated that dye penetration resulted in the expected coloration visually and/or spectroscopically and that dye was detectable for a given drug product formulation. When test articles were filled with MilliQ water alone no colouration or absorbance at 660nm was detected. When the same test articles were then filled with the methylene blue dye at a concentration of ~1.2 microMolar a blue dye coloration was observed and an absorbance at 660nm was detected with circa ~0.010 Absorbance units complying with the ICH guidelines for specificity.

Detector response linearity was demonstrated from 0.78 to 12.5 microMoles per Litre methylene blue (MB) in MilliQ water with R^2 values (correlation coefficient) of 0.997, 0.993, 0.993, 0.993, 0.995, 0.997 (n=6) with two operators. Slopes from calibration curves were determined at 0.0094, 0.0097, 0.01, 0.0091, 0.0098 and 0.0098 without forcing data through the origin. All linearities were performed by the same two operators over five days. Interday precision and accuracy were performed over five days (n=6) with replicate readings (n=3) at a concentration of 6.25 microMolar MB. The mean absorbance at 660nm for the precision standard at 62.5 microMolar concentration was 0.056±0.002 mAb (±StdDev) with relative standard deviations (RSDs) not greater than 3.5% at this concentration level. An example of a linearity performed is provided below in figure 1S below.

File: Supplementary materials

Figure 1S. Example linear plot of methylene blue (MB) dye absorbance at 660nm versus concentration of methylene blue (MB) dye in MilliQ water (microMolar).



The limit of detection (LOD) was calculated in accordance with ICH Q2 (R1) section 6.3 using equation 1 below and limit of quantitation was calculated using equation 2 below.

Equation 1: LOD = (3.3) *(A)/(B)

Equation 2: LOQ = (10) *(A)/(B)

Where A is the standard deviation of intercept for n=6 linearity determinations and numerically equal to 0.01.

Where B is the slope of the linear correlation and has a numerical value of 0.010 mAu micro Moles^{-1} Litres.

The average LOD over six validation runs was calculated to be 0.43 microMolar (equivalent to a dilution of 1:30,000 of the working MB solution) and the average LOQ over the same six validation runs was calculated to be 1.29 microMolar (equivalent to a dilution of 1:10,000 of the working MB solution.

File: Supplementary materials

References

1. Guidance on the Validation of Pharmaceutical Quality Control Analytical Methods - NHS Pharmaceutical Quality Assurance Committee March 2005. www.medicinesresources.nhs.uk/en/Communities/NHS/UKQAInfoZone

2. ICH Q2(R1) Validation of Analytical Procedures: Methodology www.ich.org/products/guidelines.html