

SOP: SP030**Percent SDS Determination in an Aqueous Solution****Materials and Reagents:**

1. Sample to assay
2. Standards for curve (note 1)
3. Pipetman, P-10
4. Pipet tips, 10 μ l
5. Eppendorf tube, 0.65 ml
6. Pipetman, P-200
7. Pipet tips, 200 μ l
8. Methylene blue solution (note 2)
9. Glass capillary pipettor, 50 μ l
10. Glass pipet, 50 μ l
11. Chloroform, HPLC-grade
12. Vortexer
13. Eppendorf centrifuge
14. 96 well plate
15. Plate reader, with 655 nm filter

Protocol:

1. ____ Using P-10 pipetman and tip, add 10 μ l of sample or standard to a 0.65 ml eppendorf tube.
2. ____ Using P-200 pipetman and tip, add 100 μ l of methylene blue solution to each eppendorf tube.
3. ____ Using a 50 μ l glass capillary pipettor, add 20 μ l of chloroform to each eppendorf tube.
4. ____ Close caps on all tubes and vortex vigorously.
5. ____ Centrifuge at 3000 x g, room temperature for 5 minutes.
6. ____ Using P-200 pipetman and tip, remove 100 μ l of aqueous (top) layer from each sample and standard and transfer to 96-well plate.
7. ____ Put plate in plate reader and read absorbance at 655 nm.
8. ____ Plot square root of SDS standards against square root of O.D.655 correlation coefficient and graph residuals linear regression. Interpolate sample SDS by squaring percent SDS on graph.

Notes:

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| 1. Standards to use: | Appearance of standard: |
| 0.1% SDS | CHCl ₃ layer is darker blue and clear, no visible granules. Only a slight color in aqueous layer. |
| 0.05% SDS | CHCl ₃ is dark blue and clear, no granules. Aqueous layer very light blue. |
| 0.01% SDS | Similar to 0.05% SDS. |
| 0.005% SDS | Similar to 0.01% SDS. |
| 0.0025% SDS | Aqueous phase a bit more color, both layers are clear. |
| 0.001% SDS | Aqueous phase a bit more color, both layers are clear. |
| 0.00075% SDS | Aqueous layer getting darker blue, CHCl ₃ getting lighter, both clear. |
| 0.0005% SDS | CHCl ₃ layer is very light aqua in color and clear. Aqueous layer is blue. |
| 0.00025% SDS | Both layers are about the same color, light aqua. Both clear. |
| 0.0001% SDS | CHCl ₃ layer is almost colorless, aqueous layer back to original color. |
| 0.00005% SDS | CHCl ₃ layer is colorless and clear. |
2. To make methylene blue solution: 12 mg/L in 0.01 M HCl with 0.02% NaN₃.

Reference:

Mukerjee. Percent SDS Determination in an Aqueous Solution. *Analytical Chemistry*. (28) 1956.