

Comparison of linearity and slope of the standard curves of two different chondroitin sulfate reference materials in dimethylmethylene blue assay

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Introduction

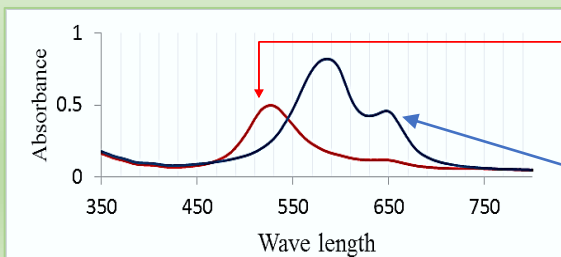
Dimethylmethylene blue assay is used to quantify sulfated glycosaminoglycans in urine which is a diagnostic biomarker of mucopolysaccharidosis.

The objective of our study was to compare the linearity and slope of the standard curves of two different standards in dimethylmethylene blue assay;

- chondroitin sulfate from bovine trachea (with high degree of polymerization).
- chondroitin sulfate oligosaccharide with degree of polymerization of 12 (12DP).

Methodology

- [100 µg/ml] Standard stock solutions and serial dilutions were prepared using the two types of chondroitin sulfate standards.
- The assay was conducted in duplicates, using three standard-to-dye volume (µl) ratios (50:150, 20:180 and 10:190) on 96-well microplates.
- The absorbance was read at 520 nm, using a microplate photometer.



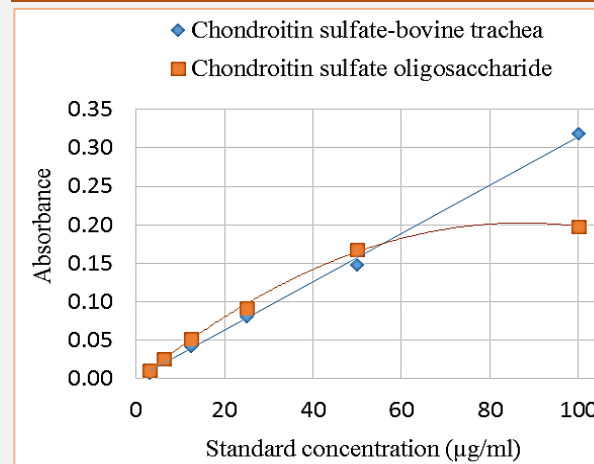
Absorbance spectrum of **chondroitin sulfate-dimethylmethylene blue complex** (*Absorbance maxima at 520 nm)

Absorbance spectrum of **dimethylmethylene blue**

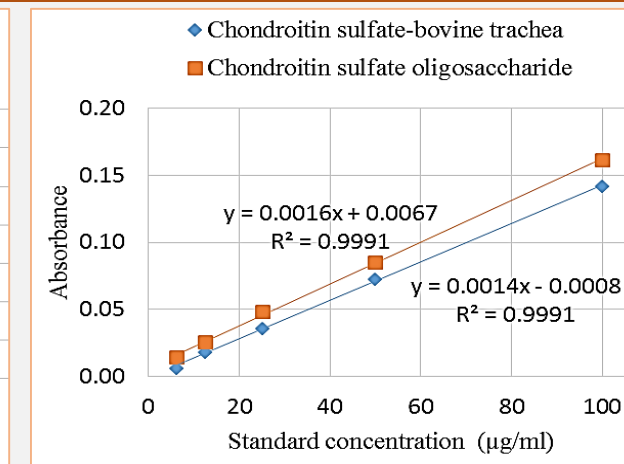
Acknowledgments:

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Results



Standard curve at 20:180 standard-to-dye ratio



Standard curve at 10:190 standard-to-dye ratio

Calibration curves for both standards at 50:150 standard-to-dye ratio were non-linear.

Slopes of the standard curves (in terms of percent concentration in reaction volume) at 10:190 standard-to-dye ratio;

- Chondroitin sulfate-bovine trachea = (0.0287 [g/100ml]⁻¹)
- Chondroitin sulfate oligosaccharide = (0.0312 [g/100ml]⁻¹)

Conclusions

Degree of polymerization and source of the glycosaminoglycan reference materials may affect linearity and slope of the standard curves and hence parallelism and commutability of the reference materials in dimethylmethylene blue assay.