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

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Background: Dimethylmethylene blue assay is used to quantify sulfated glycosaminoglycans in urine. Chondroitin sulfate polysaccharides have been routinely used as the reference materials for the assay, although majority of urinary glycosaminoglycans are oligosaccharides.

Objective: 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 vs. chondroitin sulfate oligosaccharide with degree of polymerization of 12.

Method: [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 (Multiskan FC).

Results: At 20:180 standard-to-dye ratio, the standard curve of chondroitin sulfate from bovine trachea was linear up to standard concentration of 100 µg/ml, while the oligosaccharide showed linearity only up to 25 µg/ml. At 10:190 standard-to-dye ratio, both standard curves were linear up 100 µg/ml, but the slope of the standard curve of bovine chondroitin sulfate (0.0287 [g/100ml]⁻¹) was slightly lower than that of the chondroitin sulfate oligosaccharide (0.0312 [g/100ml]⁻¹).

Conclusion: 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.

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