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Applicability of anthrone dyeing microtiter method for screening of hydroxyethyl starch and dextran – a deeper insight

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Abstract

Plasma volume expanders belong to the list of prohibited substances and methods in sports of World Anti-Doping Agency (WADA) and the purpose of this work was to find out the suitability of the anthrone dyeing microtiter method for routine doping control analysis of hydroxyethyl starch (HES) and dextran.

The performance of the method was evaluated with regard to linearity, matrix influence, accuracy and repeatability. The method was proven accurate, repeatable and linear within the range 0-900 µg/ml of HES and dextran when tested with spiked pooled urine. However, a remarkable difference in color and intensity between different urine samples was noticed, which causes dispersion and complicates the setting of a threshold in routine measurements. Furthermore, the dispersion caused by individual urine samples could not be corrected even with sample specific references and due to these specificity issues, mass spectrometric methods remain as methods of choice also for initial testing procedures.

Introduction

Plasma volume expanders belong to the list of prohibited substances and methods in sports of World Anti-Doping Agency (WADA). Different kinds of rapid colorimetric microtiter and other colour dependent screening methods have been proposed as the initial testing procedure for polysaccharide-based plasma volume expanders, such as hydroxyethyl starch (HES) and dextran [1-3]. The purpose of this work was to examine the applicability of the anthrone dyeing microtiter method for routine doping control analysis.

Experimental

Anthrone dyeing procedure was carried out according to the method suggested earlier by Gutiérrez Gallego et al [1]. Sample aliquots of 10 µl were placed into a microtiter plate and 130 µl of dyeing solution mixture (anthrone:H_2SO_4, 2:1000, w:v) was added to each sample. The plate was shaken for 2 min, sealed with acetate-tape and incubated at 70^{o}C for 30 min. Absorbancies were measured with Multiskan, EX-microplate reader (Labsystems) at wavelength of 620 nm.

The performance of the method was evaluated with regard to linearity, matrix effect, accuracy and repeatability. The method was calibrated by measuring HES and dextran samples spiked in pooled urine and water. A threshold 500 µg/ml of glucose was set for a suspicious sample.

Results and Discussion

The method was proven accurate and repeatable within the range 0-900 µg/ml of HES and dextran when tested with spiked pooled urine samples (Tables 1 and 2).

Table 1. Intra-day precision and accuracy statistics, n=6

	Mean (µg/ml)	RSD (%)	Bias (%)
HES 200 µg/ml	248	16,5	24,0
HES 400 µg/ml	440	5,5	10,0
HES 800 µg/ml	922	8,5	15,3
Dextran 200 µg/ml	215	26,8	7,5
Dextran 400 µg/ml	406	8,4	1,5
Dextran 800 µg/ml	830	7,6	3,8

Table 2. Inter-day precision and accuracy statistics, n=6

	Mean (µg/ml)	RSD (%)	Bias (%)
HES 200 µg/ml	212	20	6,0
HES 400 µg/ml	422	5,6	5,5
HES 800 µg/ml	898	15,3	12,3
Dextran 200 µg/ml	235	26,8	17,5
Dextran 400 µg/ml	412	13,3	3,0
Dextran 800 µg/ml	944	26,6	18,0

The method was proven linear within the range 0-900 µg/ml of HES and dextran when tested with samples spiked in pooled urine and water. However, a significant difference in curve intercepts was observed between urine and water matrix (Figure 1.), because of the colouring of urine. The results were comparable with HES and dextran.

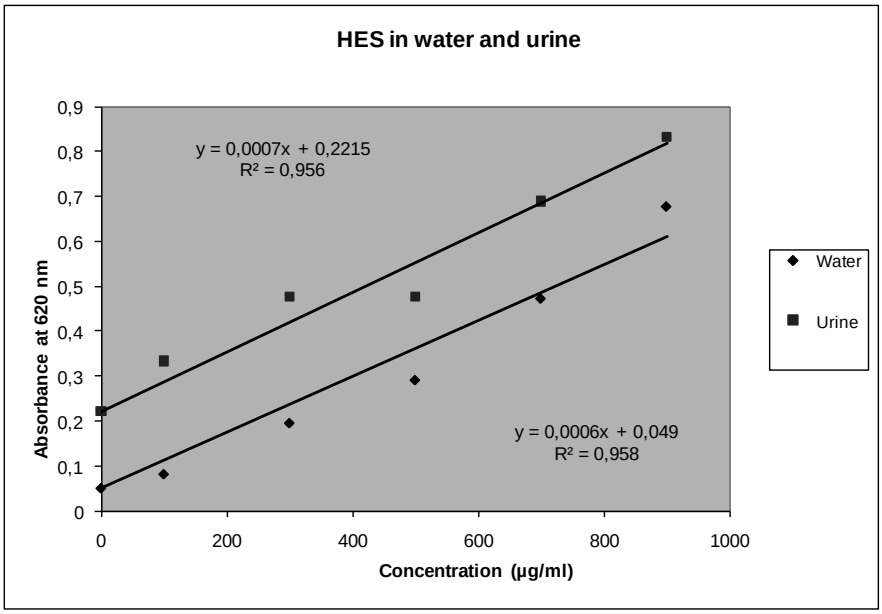

Figure 1. Calibration curves of HES in water and urine

The effect of urine matrix was even more obvious when several authentic blank urine samples containing negligible amounts of glucose were analyzed. A remarkable difference in colour and intensity was observed between individual urine samples (Figure 2.). The inter-individual dispersion complicates the setting of a threshold in routine measurements and unfortunately, could not be corrected even with sample specific references by measuring the samples with dyeing solution mixture (anthrone+sulfuric acid) and with sulfuric acid only.

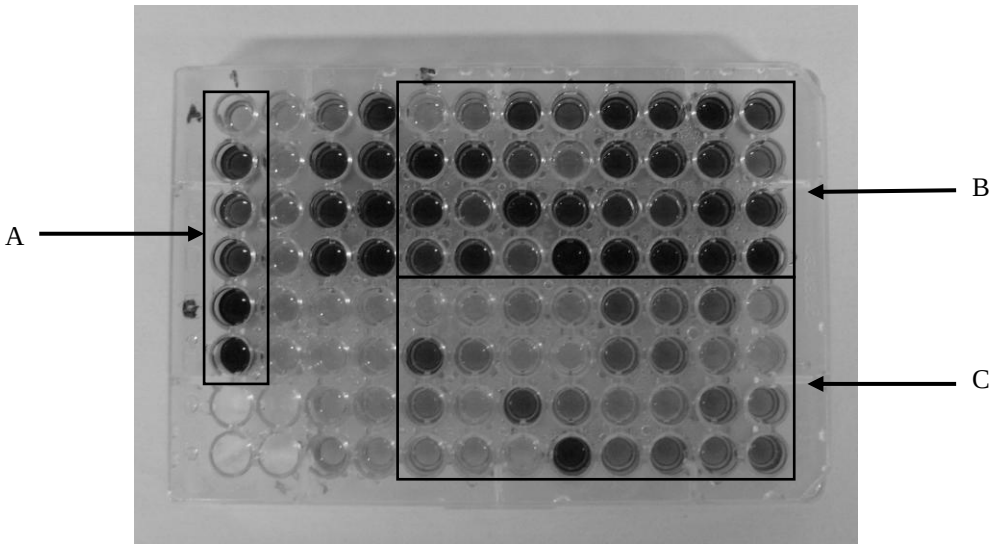

Figure 2. Microtiter plate, block A: calibrators (0, 100, 300, 500, 700 and 900 µg/ml, from top to bottom), block B: blank urine samples with dyeing solution mixture, block C: same urine samples as in block B with sulfuric acid only.

Conclusions

Despite its rapidness and easiness, the anthrone dyeing microtiter method was not easily applicable to routine screening of polysaccharide-based plasma volume expanders due to the specificity issues. Therefore, mass spectrometric approaches seem to remain as methods of choice also for initial testing procedures.

References

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