E

# APPENDIX E

*In Vitro* Testing of Twelve Heparinoid Agents on Four Haemostatic Parameters

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I hereby certify that the experimental studies described and the analyses presented in this report were conducted by me and/or my supervision.

Volker Laux

PRINT NAME

SIGNATURE

January 29th, 2004

DATE

## 1. Objectives

In vitro testing the efficacy of twelve (12) heparinoid agents on four haemostatic parameters assessing fibrin formation, using classical coagulation tests, and thrombin activtity.

## 2. Materials and methods

### 2.1 Coagulation tests thrombin time (TT), partial thromboplastin time (PTT), prothrombin time (Quick , Q), and thrombin generation

TT, PTT and Quick-test were determined using Amelung Amax CS-190 and CS-400 coagulation diagnostic analyzer and Dade-Behring reagents.

Thrombin generation measurement and evaluation was carried out using Hemker's methods (Hemker HC et al., Thrombosis and Haemostasis 2000; 83: 589-91). Reagents used were Tissue Factor (TF, Dade-Behring) HEPES-Buffer pH 7.5 and HEPES-Buffer pH 7.5 incl. BSA 60 mg/ml used for the colour reagent, DMSO (Sigma), $CaCl_2$-Buffer 1 M pH 7.5, fluorogenic colour reagent: Z-Gly-Gly-Arg-AMC (Bachem).

### 2.2 Heparinoid reagents tested

WSD and DIA fractions, from Aventis Pharma Germany, indicated furtheron, if necessary, by the following numbers:

(1) WSD Hexasaccharide
(2) WSD Octasaccharide
(3) WSD Decasaccharide
(4) WSD Dodecasaccharide
(5) WSD <Hexadecasaccharide
(6) WSD >Hexadecasaccharide

(7) DIA Hexasaccharide
(8) DIA Octasaccharide
(9) DIA Decasaccharide
(10) DIA Dodecasaccharide
(11) DIA <Hexadecasaccharide
(12) DIA >Hexadecasaccharide

Concentration of stem solution was normalized to 100 U antiXa activity/ml of heparinoid agent before use in further dilution for all substances.

PF4 (Chromatec, Germany) was delivered as a lyophilized powder and stored at –20 °C as a stem solution of 1mg/ml in $H_2O$ bidest.

## 2.3 Procedures and protocols

TT, PTT and Quick-test:

All Substances 1-12 tested were added to six final dilutions (0; 0.2; 0.5; 1; 2; 5; 10 U/ml. Platelet-poor plasma (PPP) was generated from citrated blood,(3,8 %) using single-step centrifugation (2000g, 30 min). The PPP samples were incubated with heparinoid type 1-6 (see above for 15 min. Subsequently, the determination of TT, PTT and Quick was carried out. Number of individuals was n=6.

Thrombin generation:

After generation of Platelet-rich plasma (PRP) from citrated blood,(3,8 %) using single-step centrifugation (750 g, 5 min), the PRP samples were incubated with the heparinoid reagents. Substances 1-12 tested were added to six final dilutions (0; 0.2; 0.5; 1; 2; 5; 10 U/m and incubated for 15 min. Samples for fluorometry were composed of 6,25 µl of trigger (TF dilution 10 nM, Innovin from Dade Behring, dilution 1:600, which was also used for the individual reference values), 25 µl of PRP and 6,25 µl of colour reagent. Afterwards, thrombin generation was determined using a Labsystems Fluoroskan FL-fluorometer. Fluorescence signals were further processed using ASCENT software v. 2.4.2 and Microsoft Excel 97. Number of individuals was n=6.

Curve fitting:

EC 50 calculation was done using GraphPad Prism 3.0c for nonlinear regression (ETP, PEAK) and direct graphical approximation from the curves.

Platelet factor 4 (PF 4) antagonism:

Each fraction of WSD and DIA was tested for the ability of PF4 to enhance or reduce its inhibitory effect on coagulation. The heparinoids' dose chosen was averaged (see below, 3.3). PF4 concentrations used were 0.5, 5 and 50 µg/ml.

Statistical analysis:

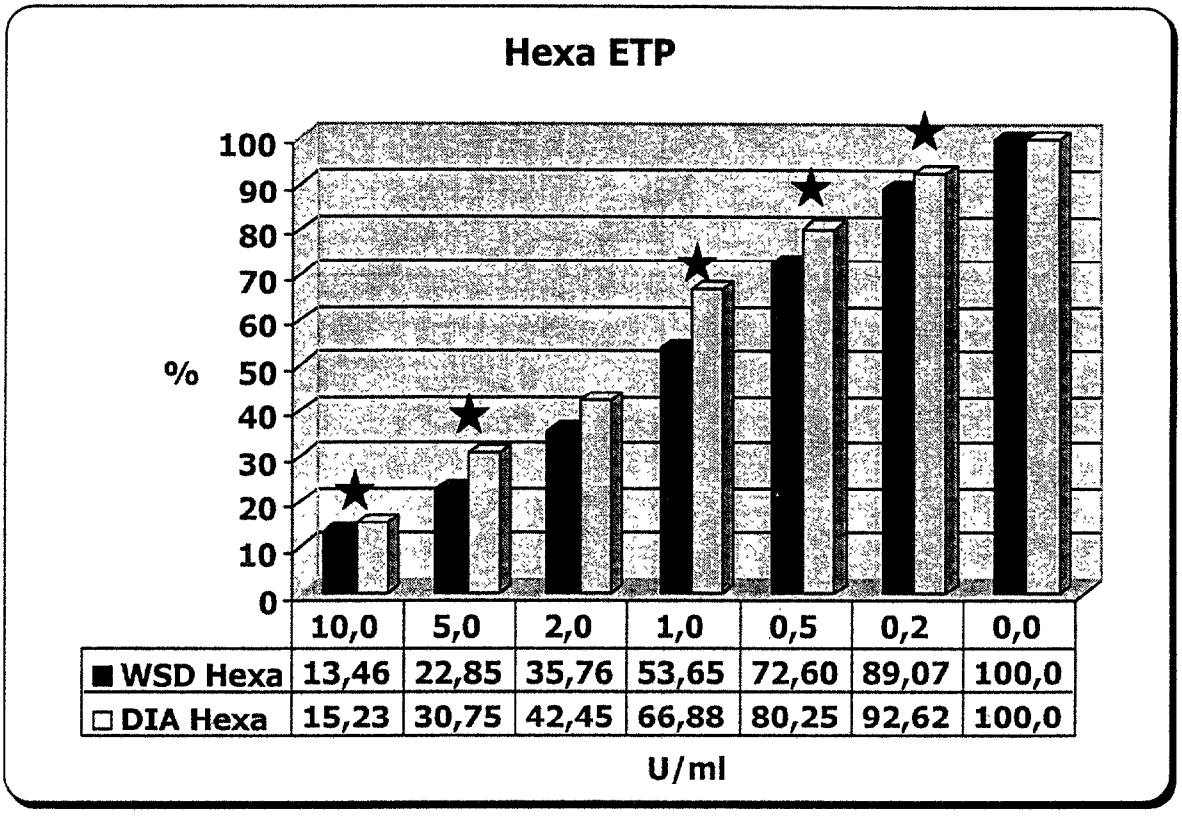
The data was tested for corresponding pairs of WSD and DIA fractions, for each concentration separately, using the paired sign test and the Wilcoxon signed rank test for non-parametric paired samples, according to the recommendations by Dr. Frank Krummenauer from the Mainz University medical statistics department.

3. Results

3.1 Dose-response curves to determine characteristics and EC 50 of heparinoid efficacy

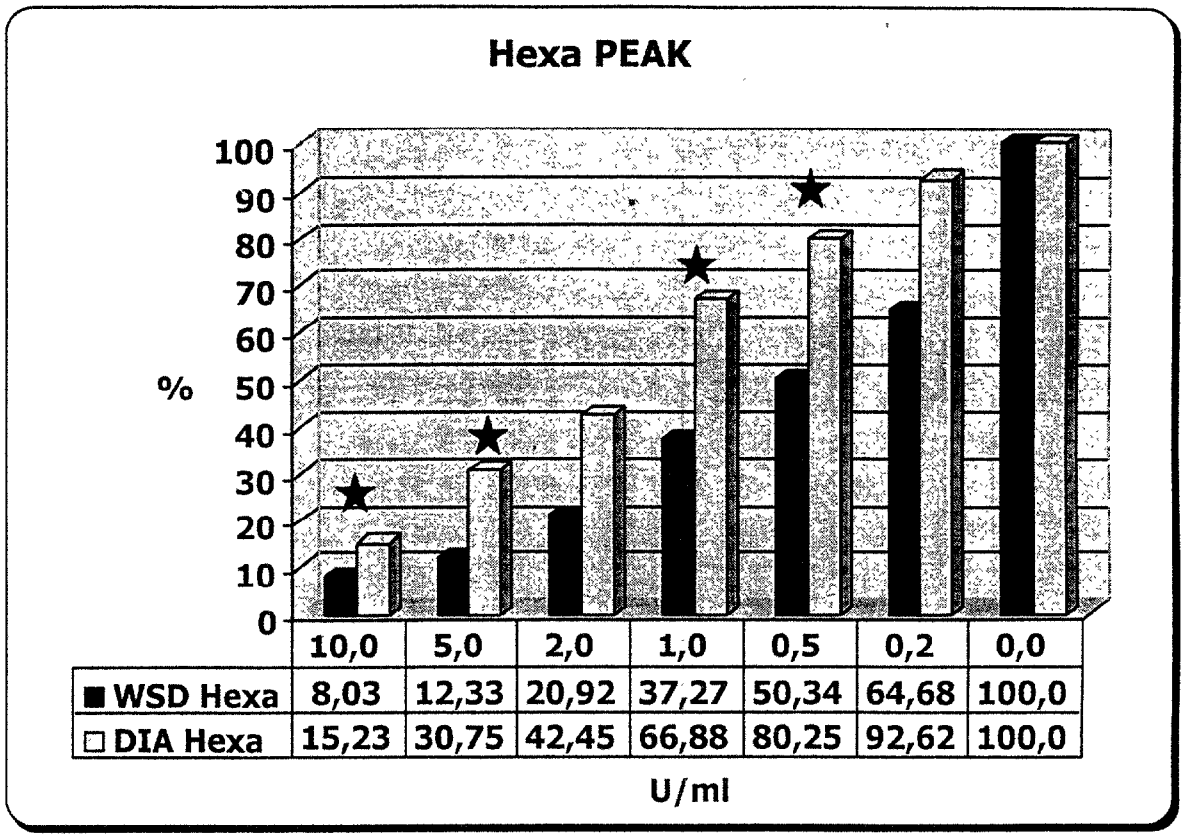
All values were normalized to percent of an individual reference value, which was taken as 100 % of individual thrombin activity, and generated using a defined stimulus (see above). Bars indicate means:

a) Hexasaccharide:



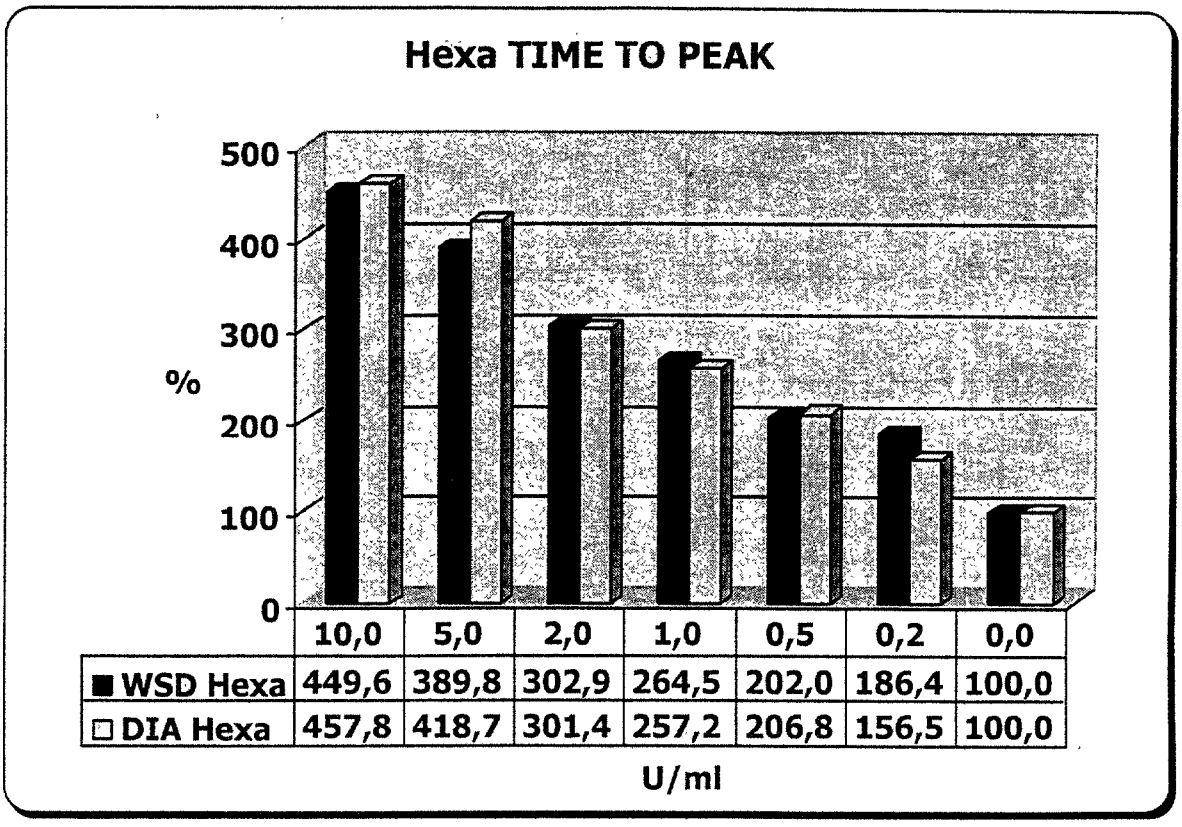

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Hexa | 13,46 | 22,85 | 35,76 | 53,65 | 72,60 | 89,07 | 100,0 |
| □ DIA Hexa | 15,23 | 30,75 | 42,45 | 66,88 | 80,25 | 92,62 | 100,0 |

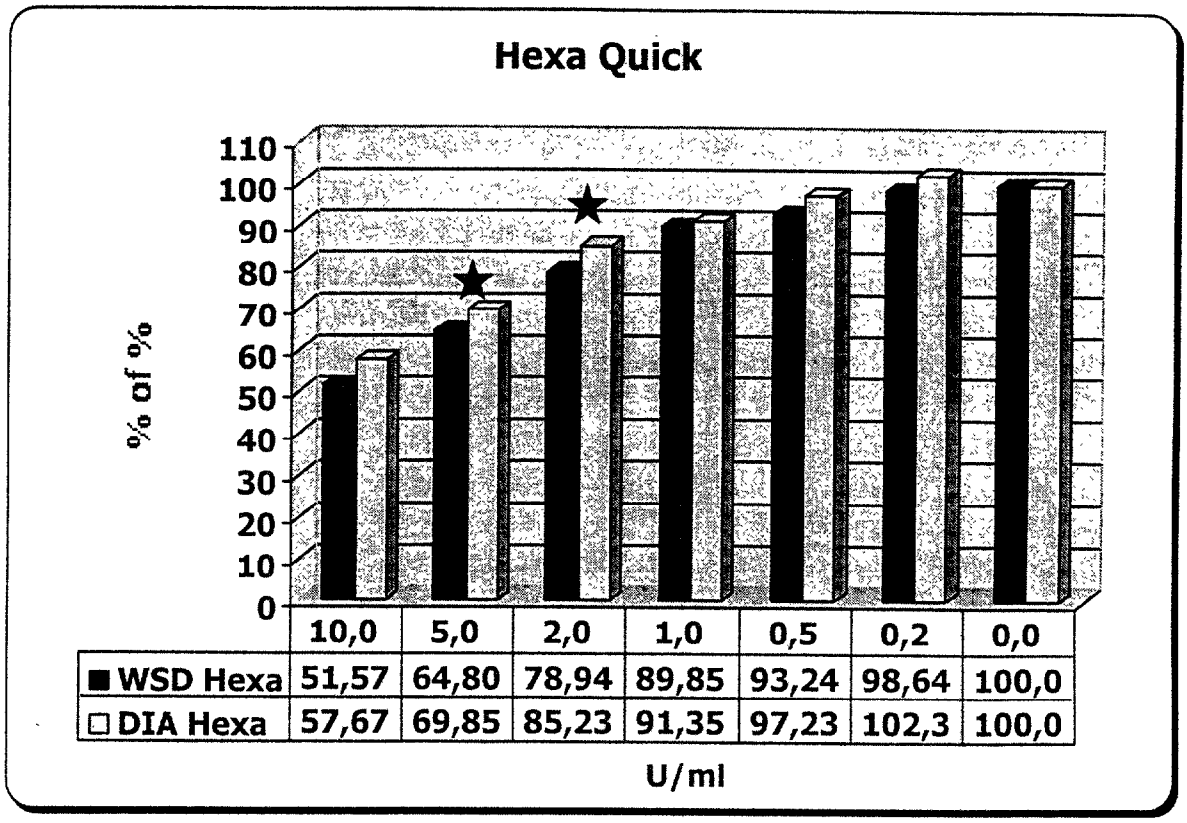
* = $p < 0,05$




| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Hexa | 8,03 | 12,33 | 20,92 | 37,27 | 50,34 | 64,68 | 100,0 |
| □ DIA Hexa | 15,23 | 30,75 | 42,45 | 66,88 | 80,25 | 92,62 | 100,0 |

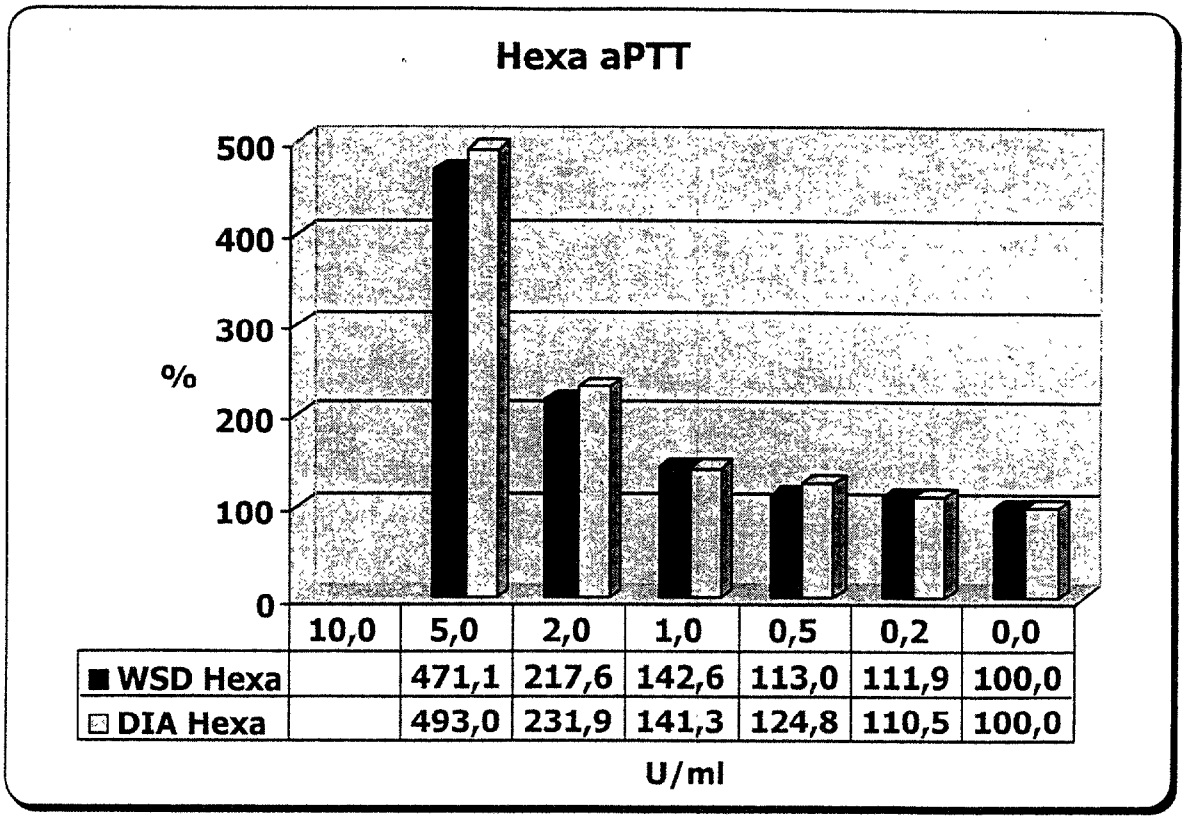
* = $p < 0,05$


Hexa TIME TO PEAK
500
400
300
200
100
0
%
10,0 5,0 2,0 1,0 0,5 0,2 0,0
WSD Hexa 449,6 389,8 302,9 264,5 202,0 186,4 100,0
DIA Hexa 457,8 418,7 301,4 257,2 206,8 156,5 100,0
U/ml

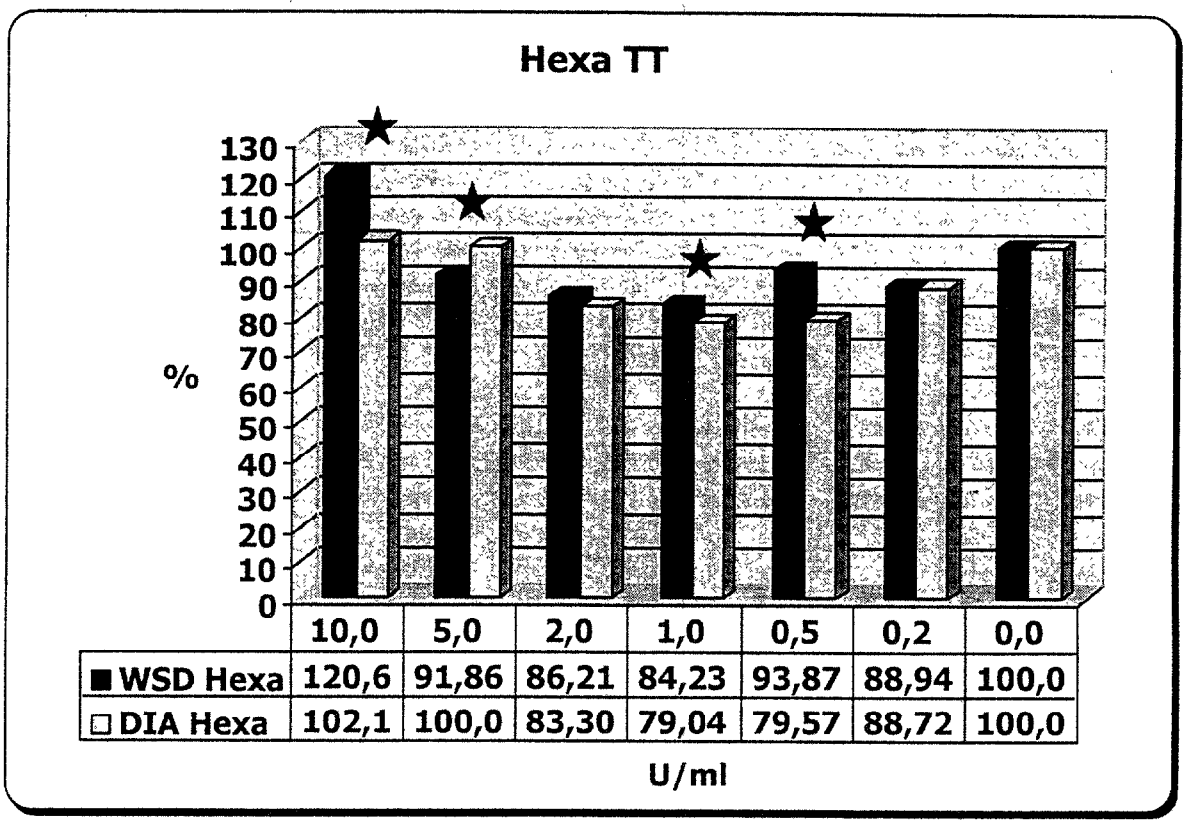



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Hexa | 51,57 | 64,80 | 78,94 | 89,85 | 93,24 | 98,64 | 100,0 |
| □ DIA Hexa | 57,67 | 69,85 | 85,23 | 91,35 | 97,23 | 102,3 | 100,0 |

* = $p < 0,05$



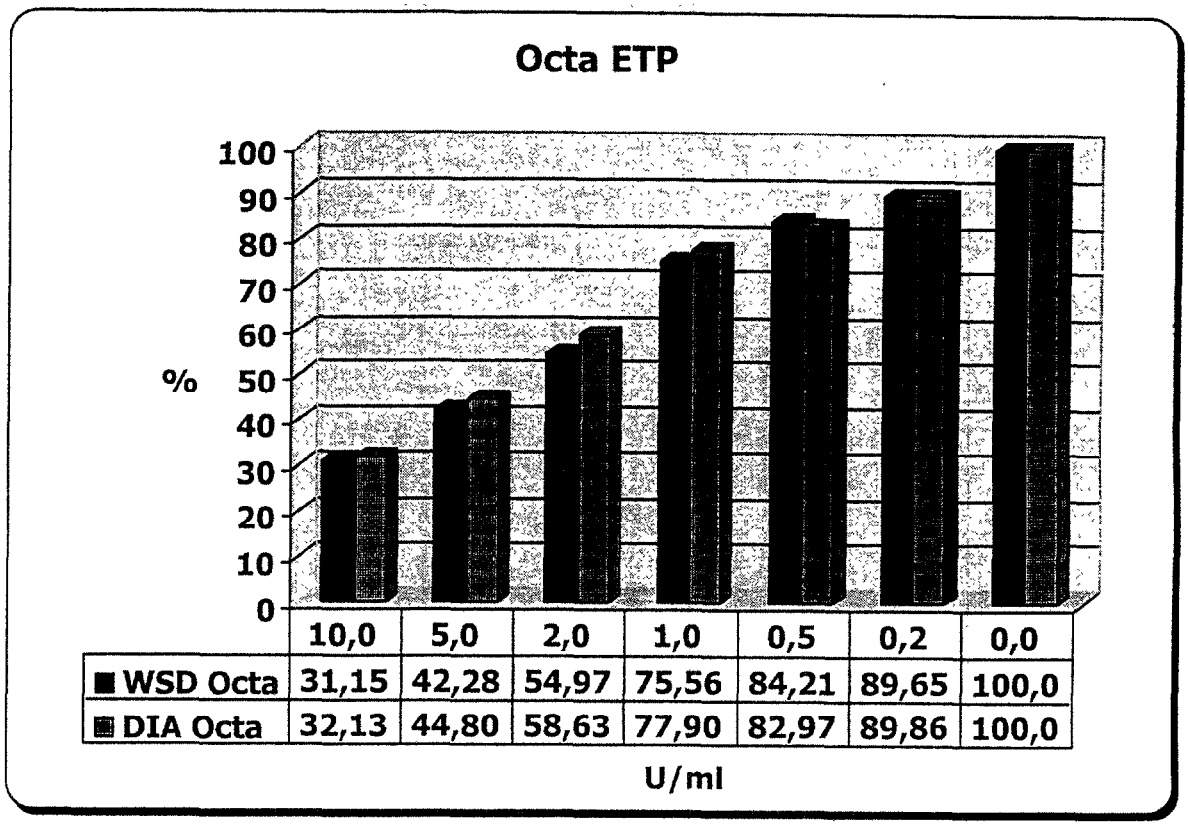

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Hexa | | 471,1 | 217,6 | 142,6 | 113,0 | 111,9 | 100,0 |
| ▨ DIA Hexa | | 493,0 | 231,9 | 141,3 | 124,8 | 110,5 | 100,0 |

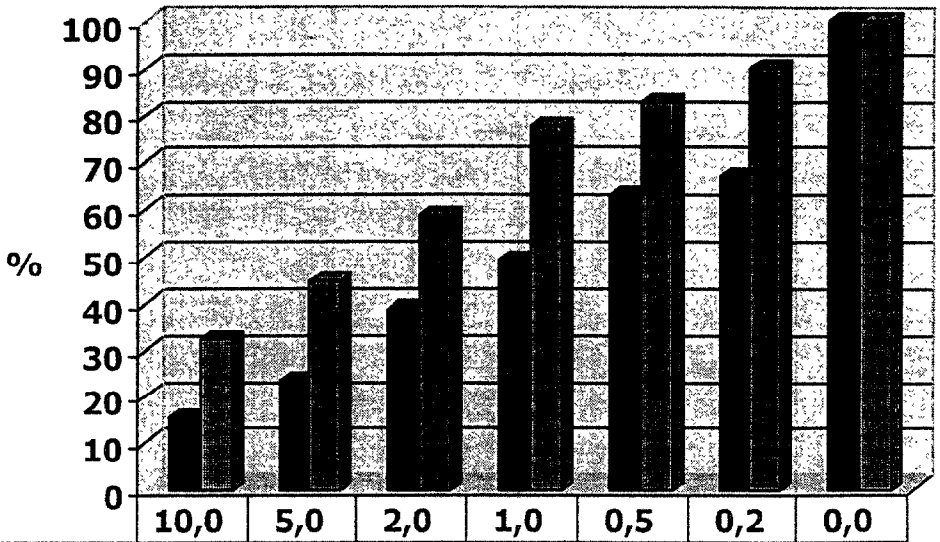



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Hexa | 120,6 | 91,86 | 86,21 | 84,23 | 93,87 | 88,94 | 100,0 |
| □ DIA Hexa | 102,1 | 100,0 | 83,30 | 79,04 | 79,57 | 88,72 | 100,0 |

* = $p < 0,05$

b) Octasaccharide:

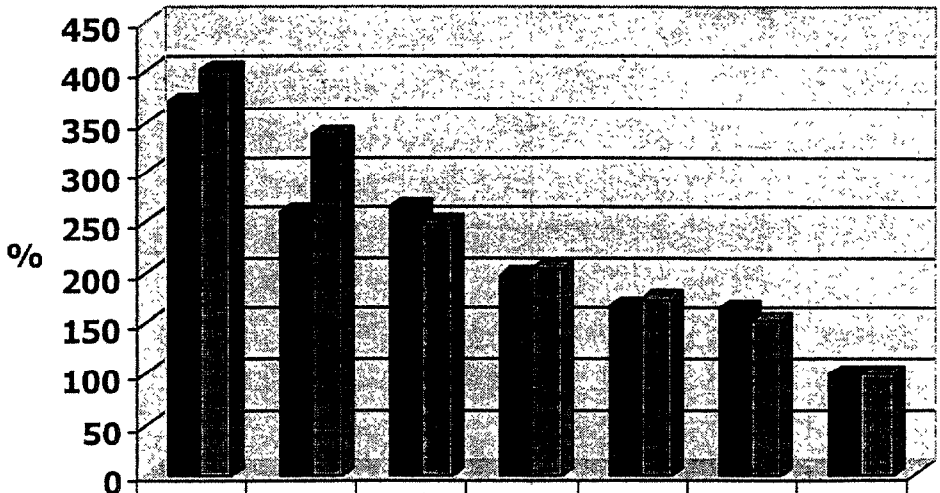





| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| WSD Octa | 15,69 | 23,38 | 38,79 | 49,04 | 63,20 | 67,19 | 100,0 |
| DIA Octa | 32,13 | 44,80 | 58,63 | 77,90 | 82,97 | 89,86 | 100,0 |

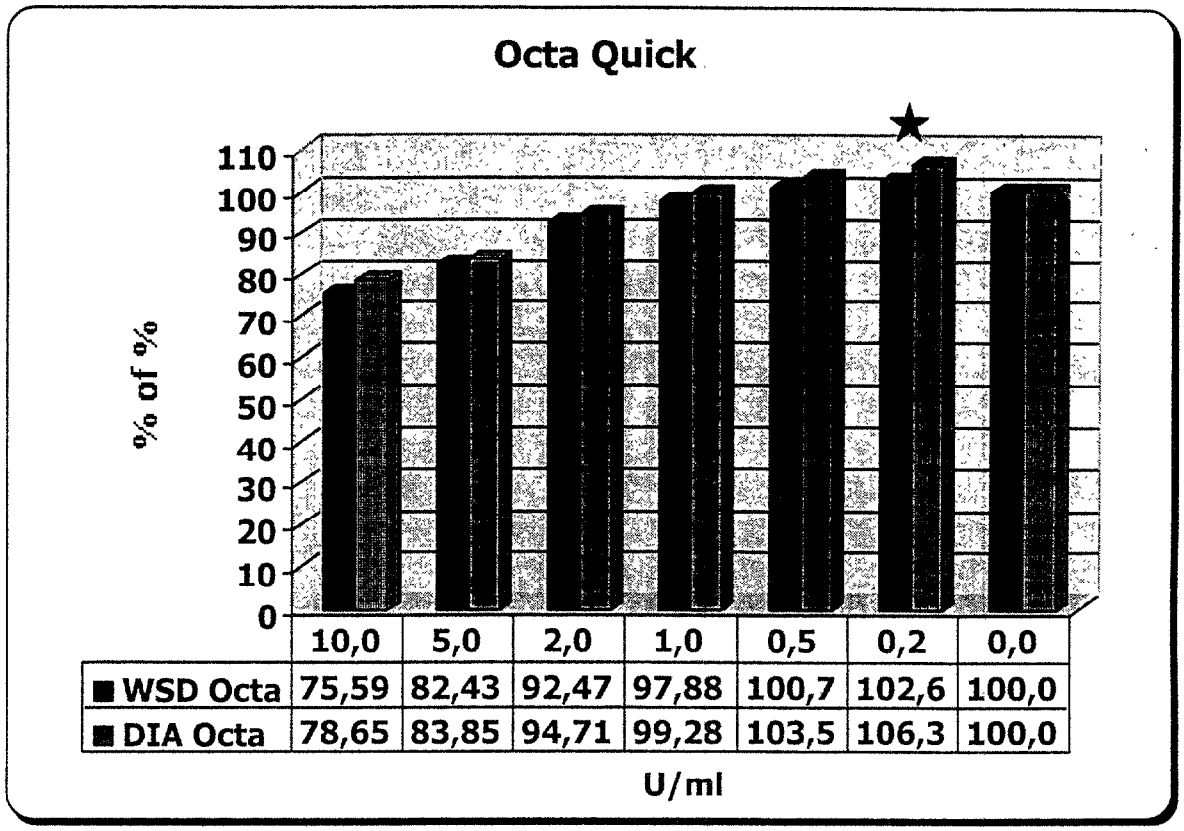
U/ml

## Octa TIME TO PEAK



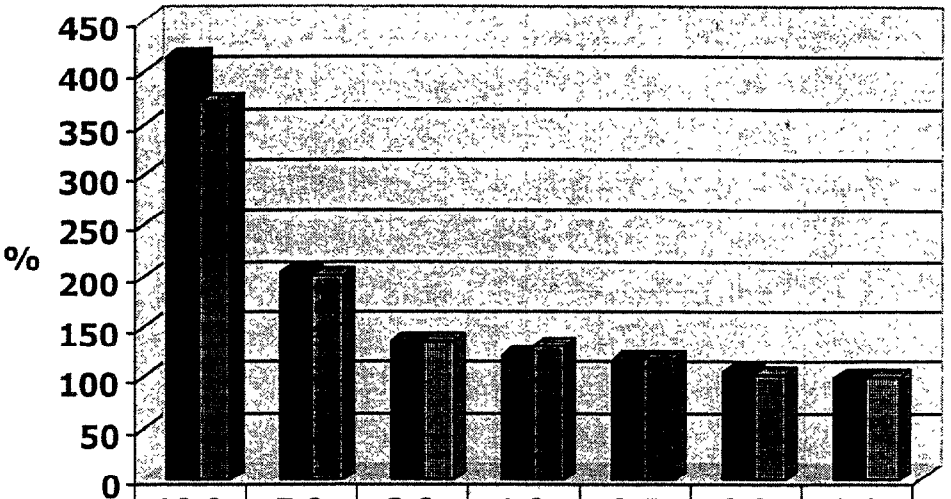

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Octa | 370,5 | 258,7 | 265,6 | 196,7 | 167,6 | 163,4 | 100,0 |
| ■ DIA Octa | 402,0 | 336,9 | 248,2 | 202,9 | 173,3 | 151,8 | 100,0 |

U/ml



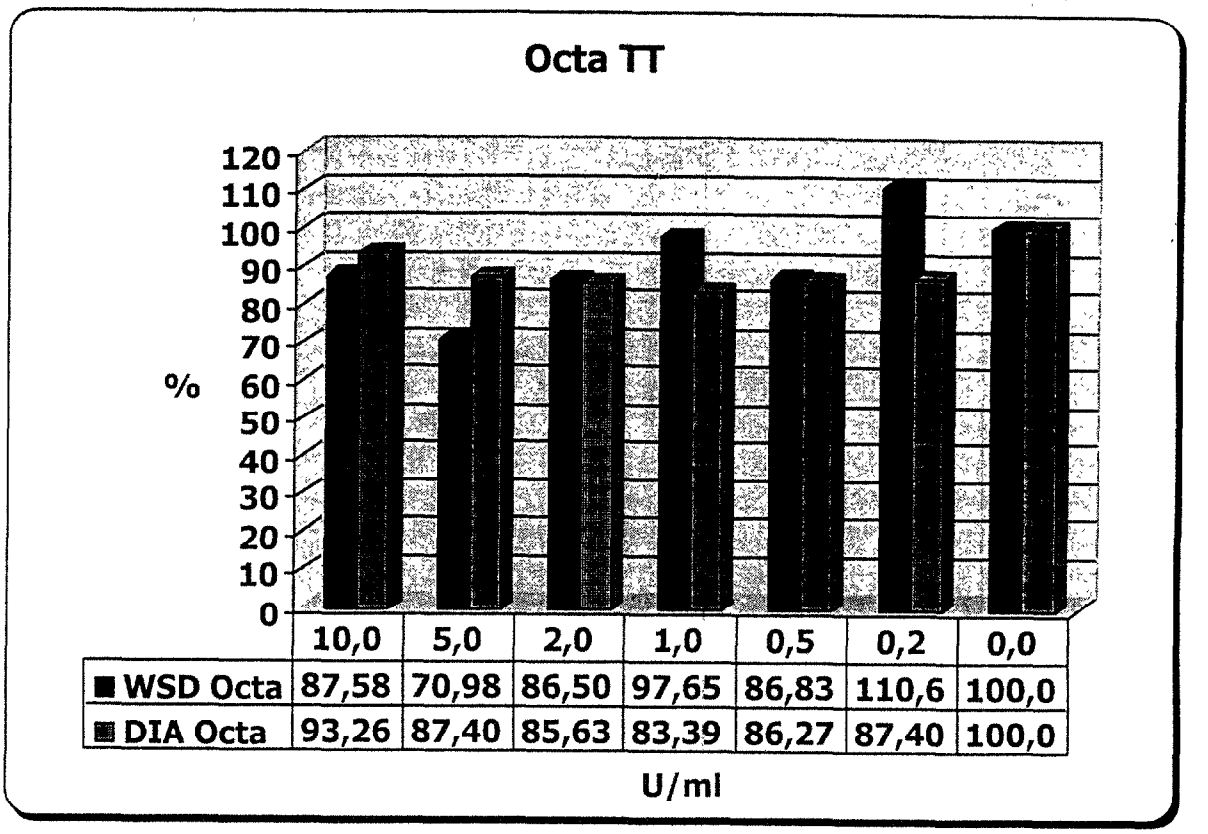

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Octa | 75,59 | 82,43 | 92,47 | 97,88 | 100,7 | 102,6 | 100,0 |
| ■ DIA Octa | 78,65 | 83,85 | 94,71 | 99,28 | 103,5 | 106,3 | 100,0 |

* = $p < 0,05$

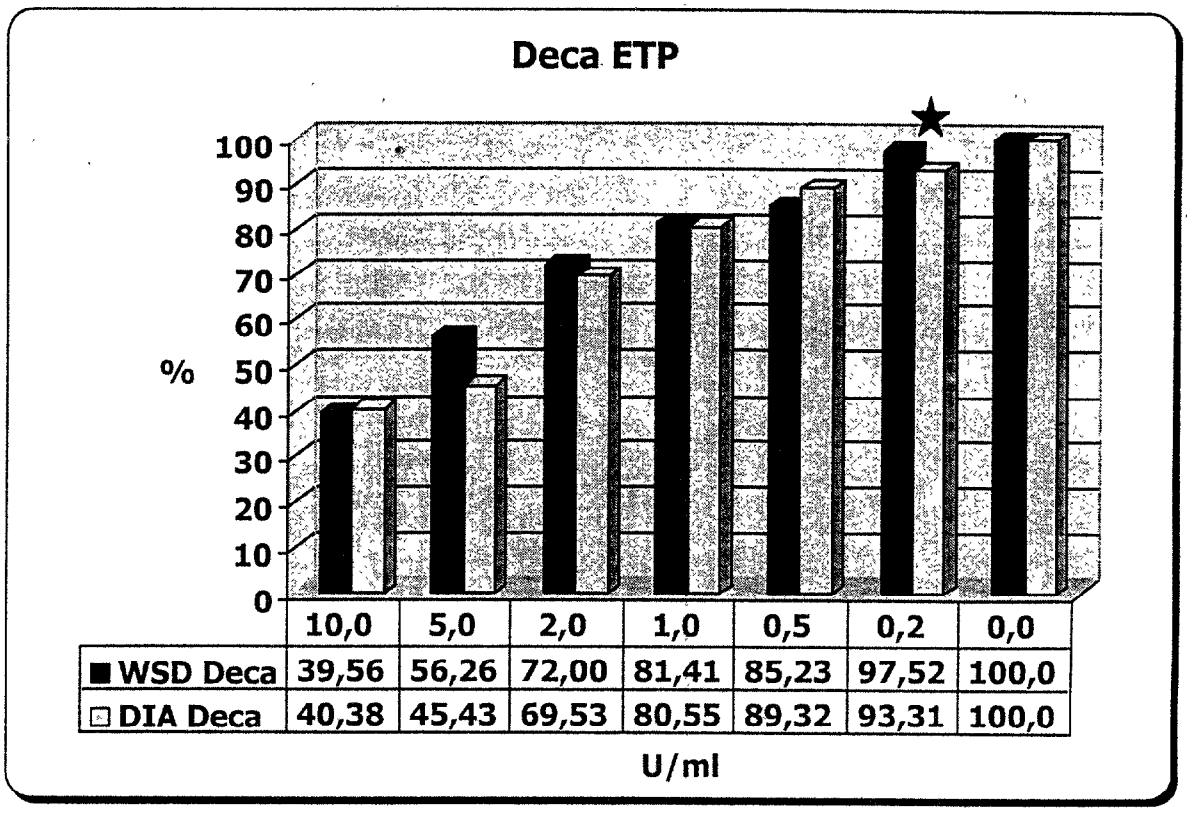



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| WSD Octa | 413,8 | 204,0 | 135,7 | 121,5 | 116,6 | 105,1 | 100,0 |
| DIA Octa | 371,2 | 200,4 | 135,8 | 131,1 | 118,0 | 101,9 | 100,0 |

U/ml

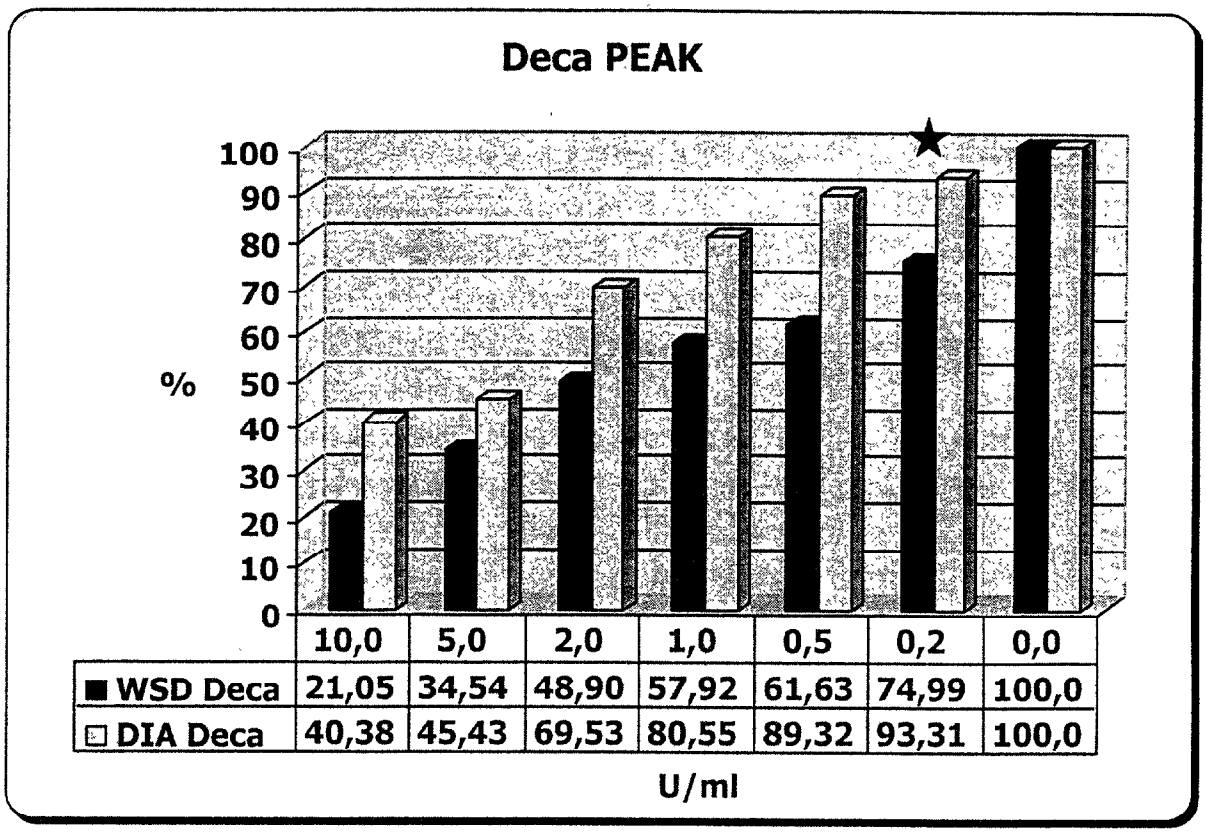

Octa TT
120
110
100
90
80
70
%
60
50
40
30
20
10
0
10,0 5,0 2,0 1,0 0,5 0,2 0,0
WSD Octa 87,58 70,98 86,50 97,65 86,83 110,6 100,0
DIA Octa 93,26 87,40 85,63 83,39 86,27 87,40 100,0
U/ml

c) Decasaccharide:



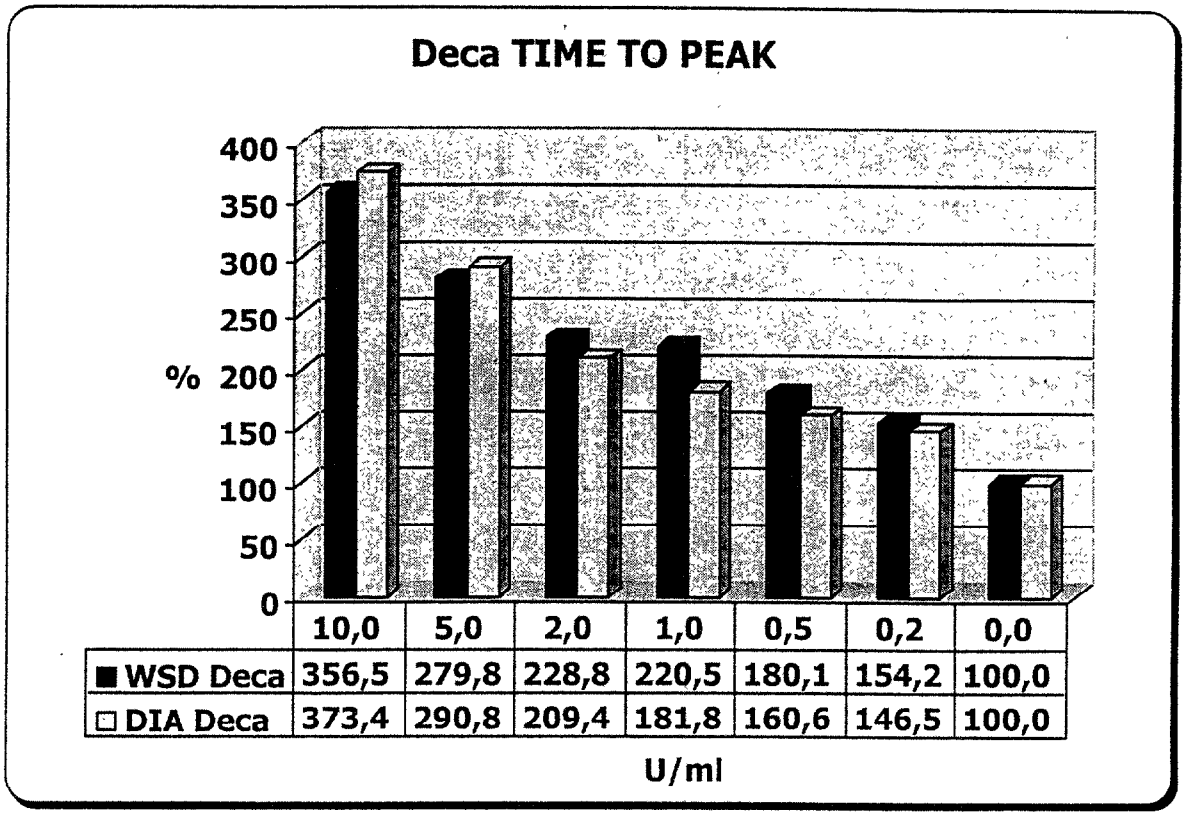

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Deca | 39,56 | 56,26 | 72,00 | 81,41 | 85,23 | 97,52 | 100,0 |
| □ DIA Deca | 40,38 | 45,43 | 69,53 | 80,55 | 89,32 | 93,31 | 100,0 |

* = $p < 0,05$

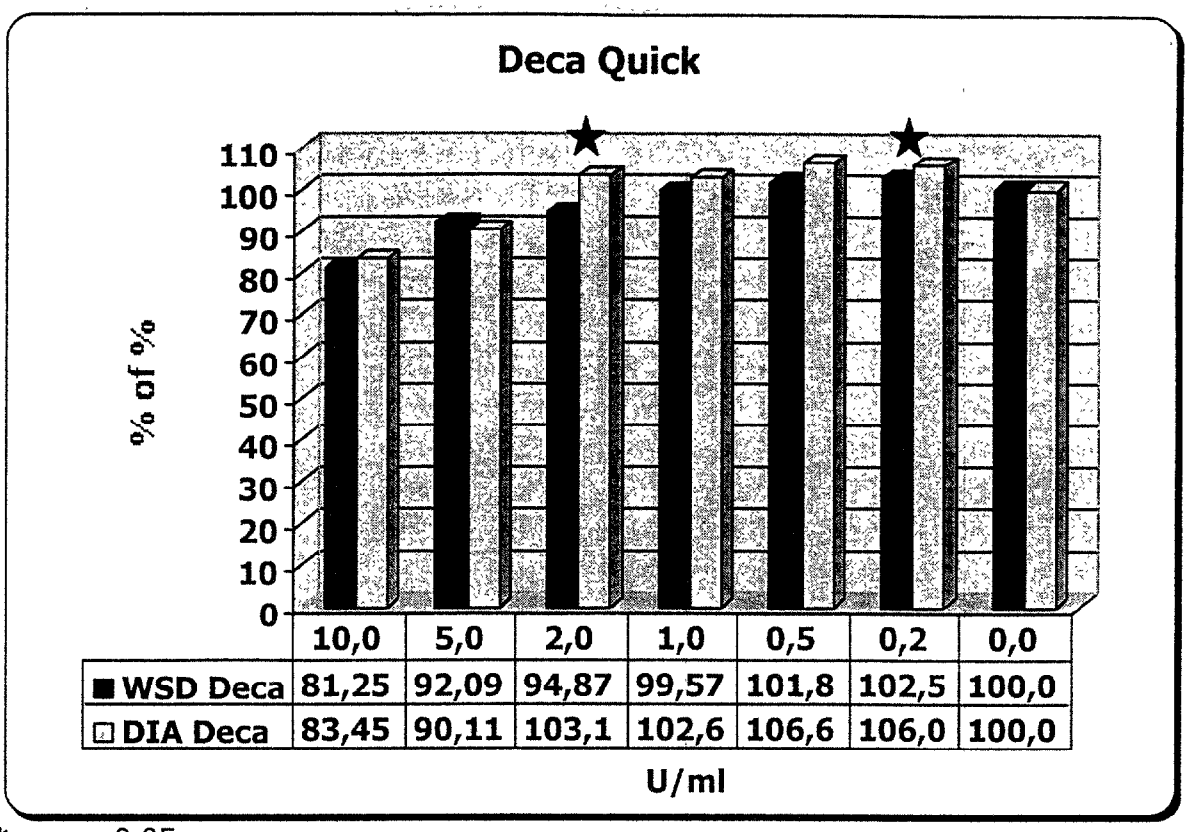



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Deca | 21,05 | 34,54 | 48,90 | 57,92 | 61,63 | 74,99 | 100,0 |
| □ DIA Deca | 40,38 | 45,43 | 69,53 | 80,55 | 89,32 | 93,31 | 100,0 |

* = $p < 0,05$



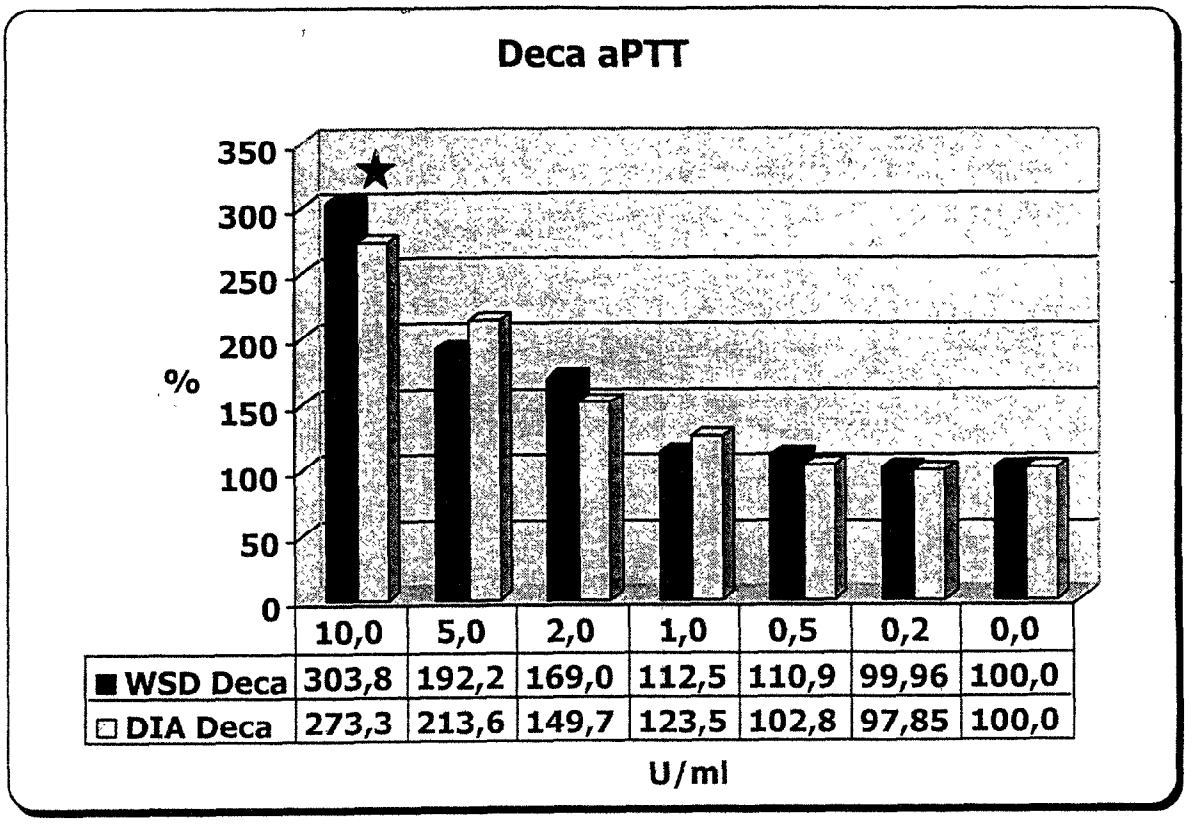

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Deca | 356,5 | 279,8 | 228,8 | 220,5 | 180,1 | 154,2 | 100,0 |
| □ DIA Deca | 373,4 | 290,8 | 209,4 | 181,8 | 160,6 | 146,5 | 100,0 |



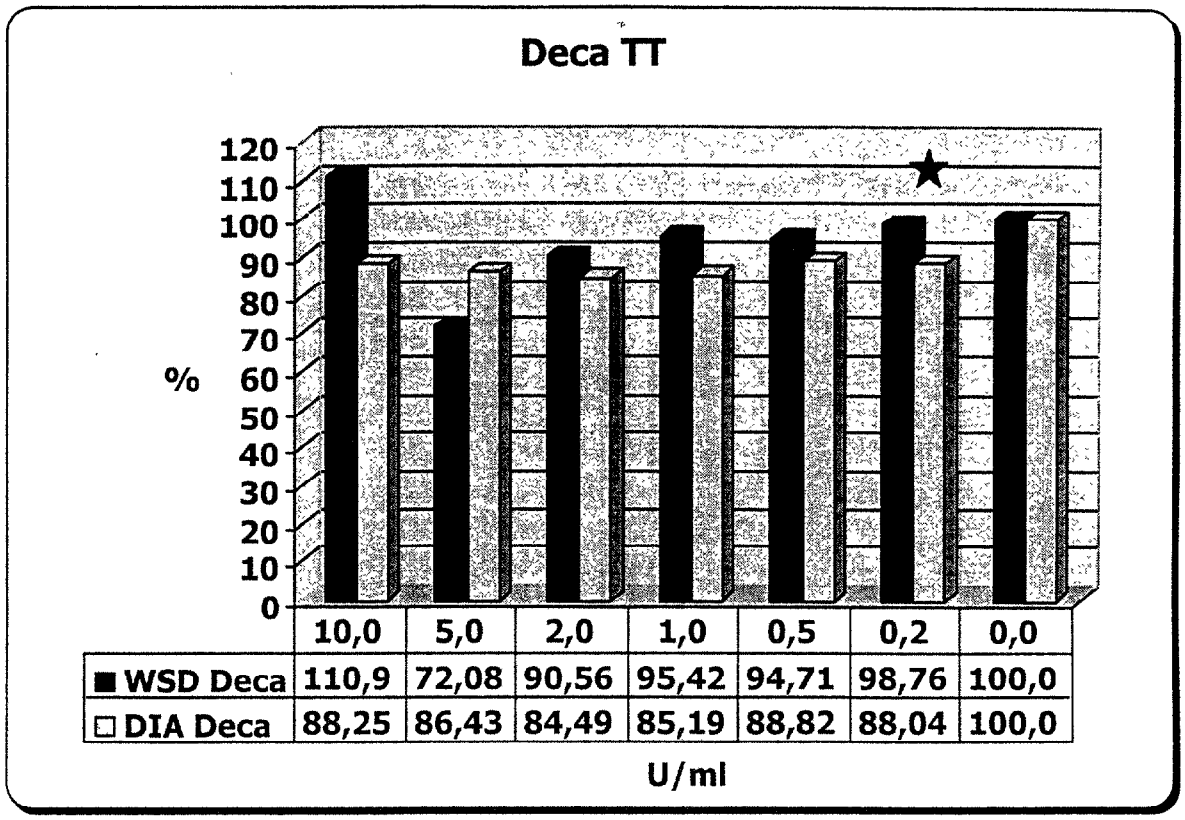

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Deca | 81,25 | 92,09 | 94,87 | 99,57 | 101,8 | 102,5 | 100,0 |
| □ DIA Deca | 83,45 | 90,11 | 103,1 | 102,6 | 106,6 | 106,0 | 100,0 |

* = $p < 0,05$

*



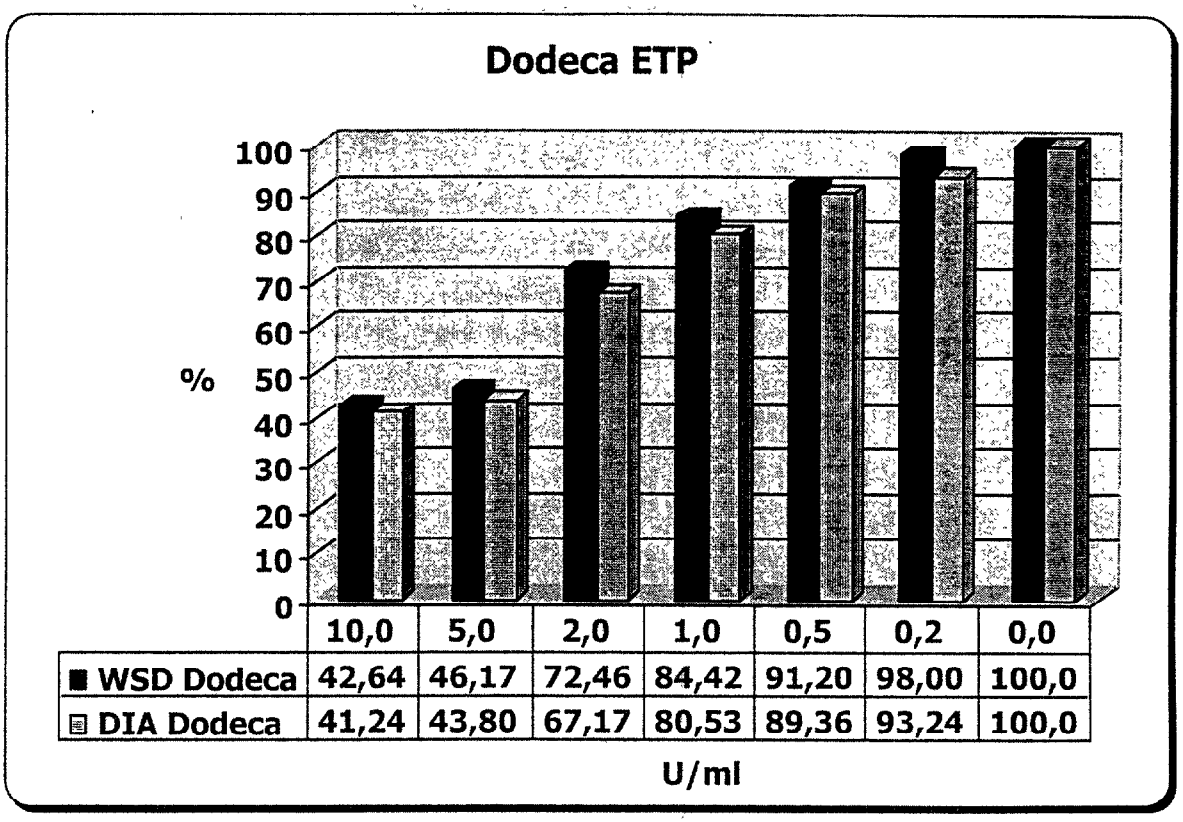

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Deca | 303,8 | 192,2 | 169,0 | 112,5 | 110,9 | 99,96 | 100,0 |
| □ DIA Deca | 273,3 | 213,6 | 149,7 | 123,5 | 102,8 | 97,85 | 100,0 |



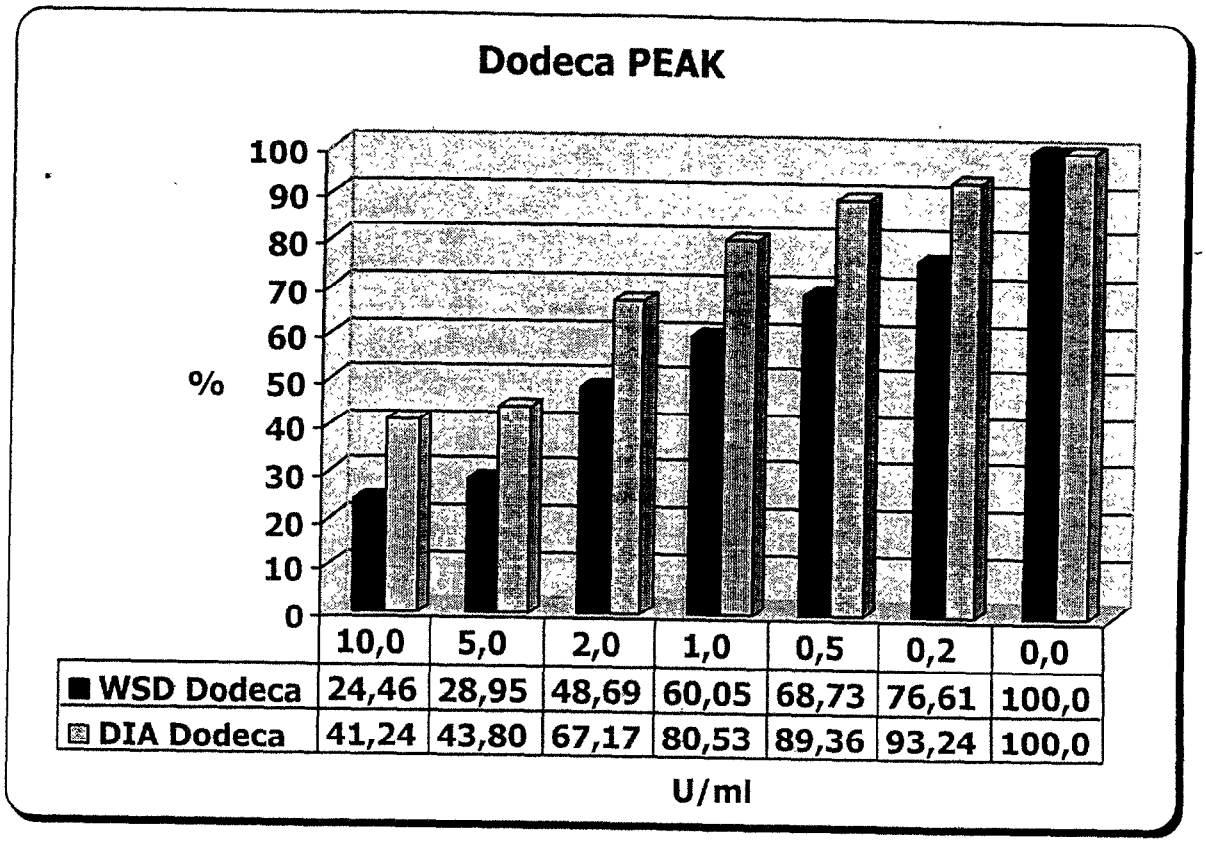

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Deca | 110,9 | 72,08 | 90,56 | 95,42 | 94,71 | 98,76 | 100,0 |
| □ DIA Deca | 88,25 | 86,43 | 84,49 | 85,19 | 88,82 | 88,04 | 100,0 |

* = $p < 0,05$

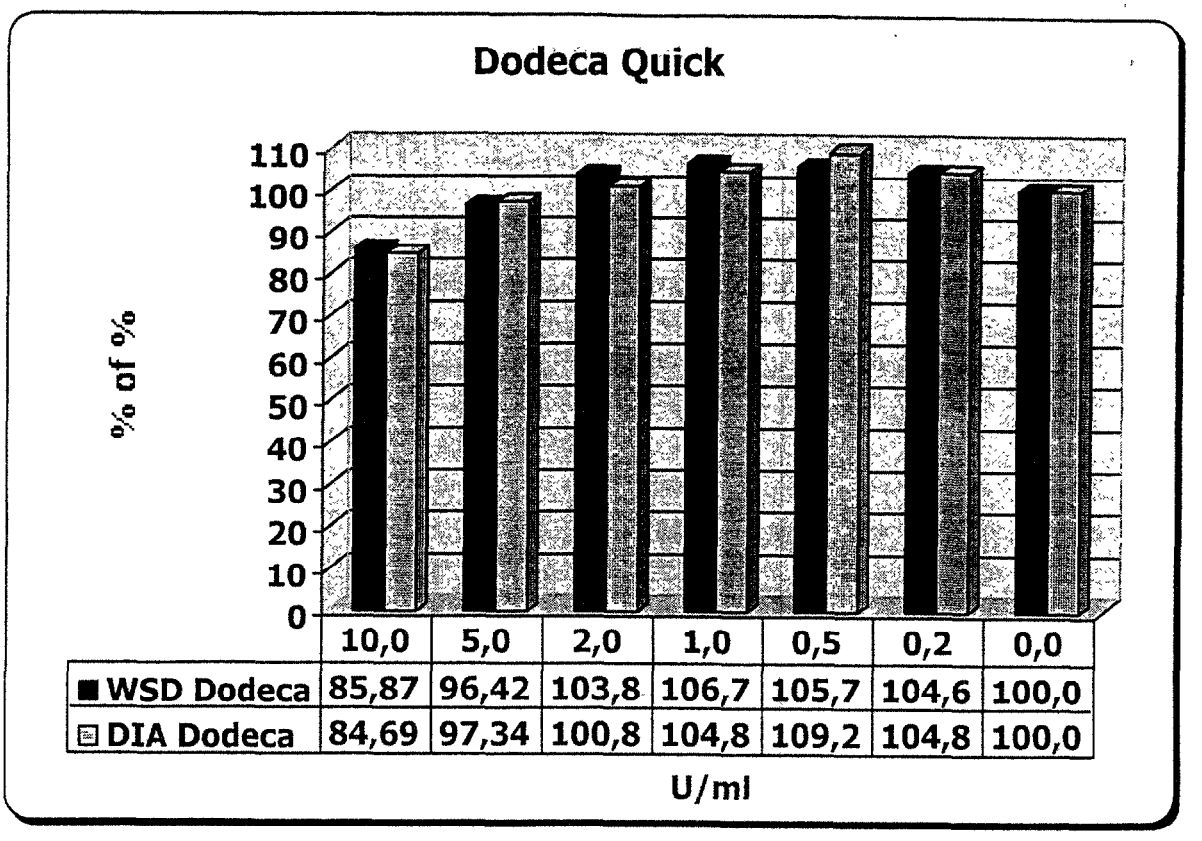
d) Dodecasaccharide:



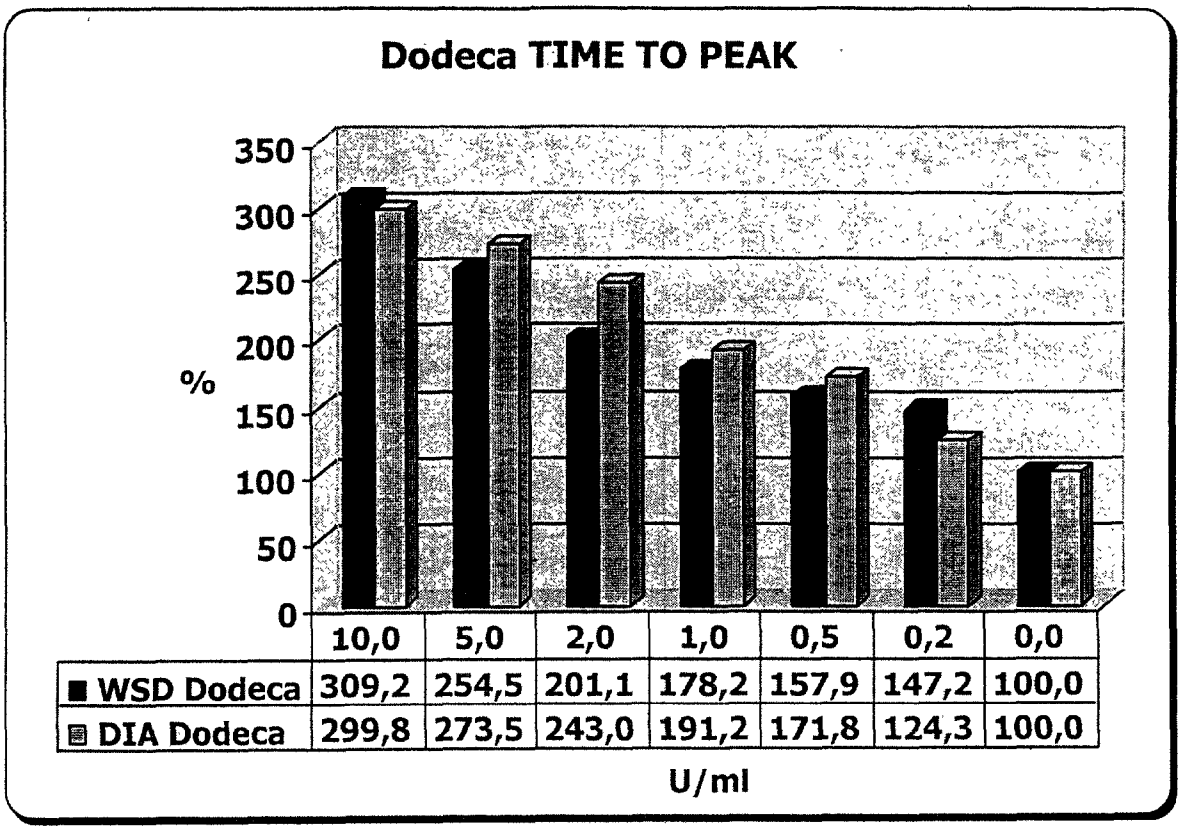

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| WSD Dodeca | 42,64 | 46,17 | 72,46 | 84,42 | 91,20 | 98,00 | 100,0 |
| DIA Dodeca | 41,24 | 43,80 | 67,17 | 80,53 | 89,36 | 93,24 | 100,0 |



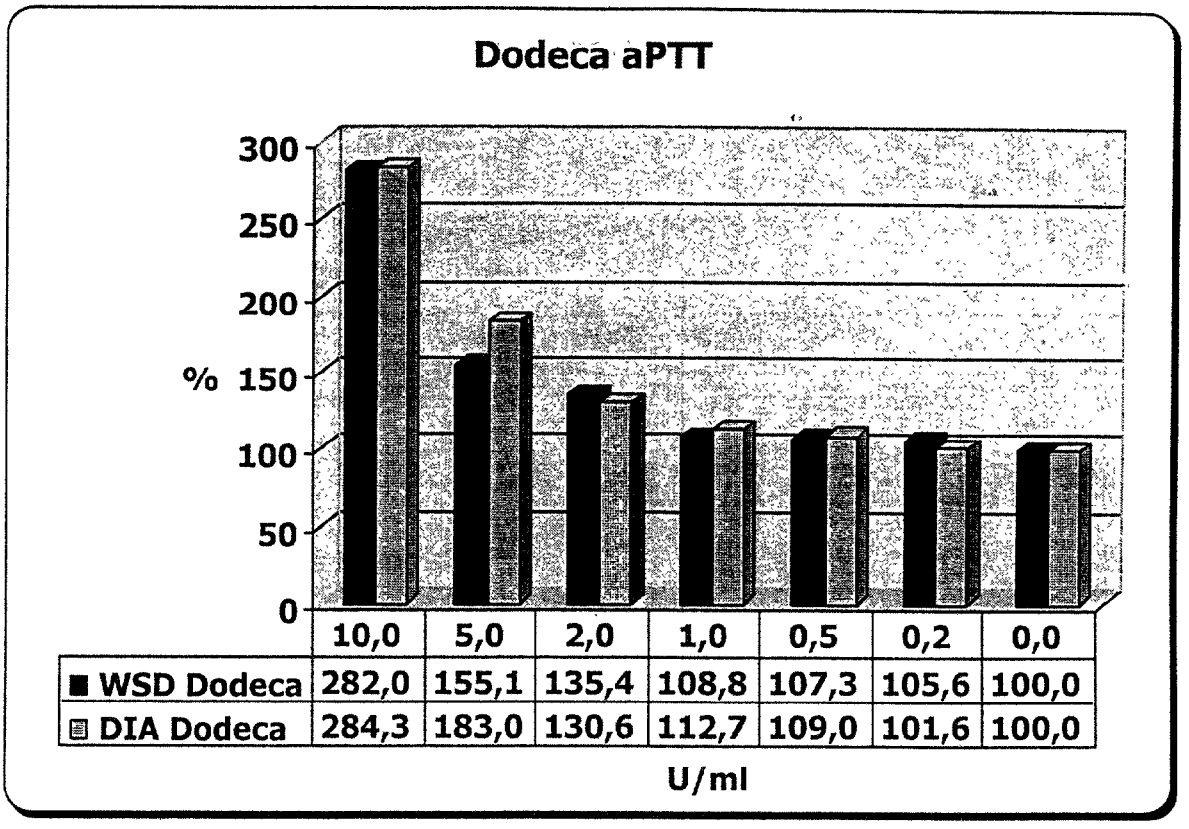

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| WSD Dodeca | 24,46 | 28,95 | 48,69 | 60,05 | 68,73 | 76,61 | 100,0 |
| DIA Dodeca | 41,24 | 43,80 | 67,17 | 80,53 | 89,36 | 93,24 | 100,0 |



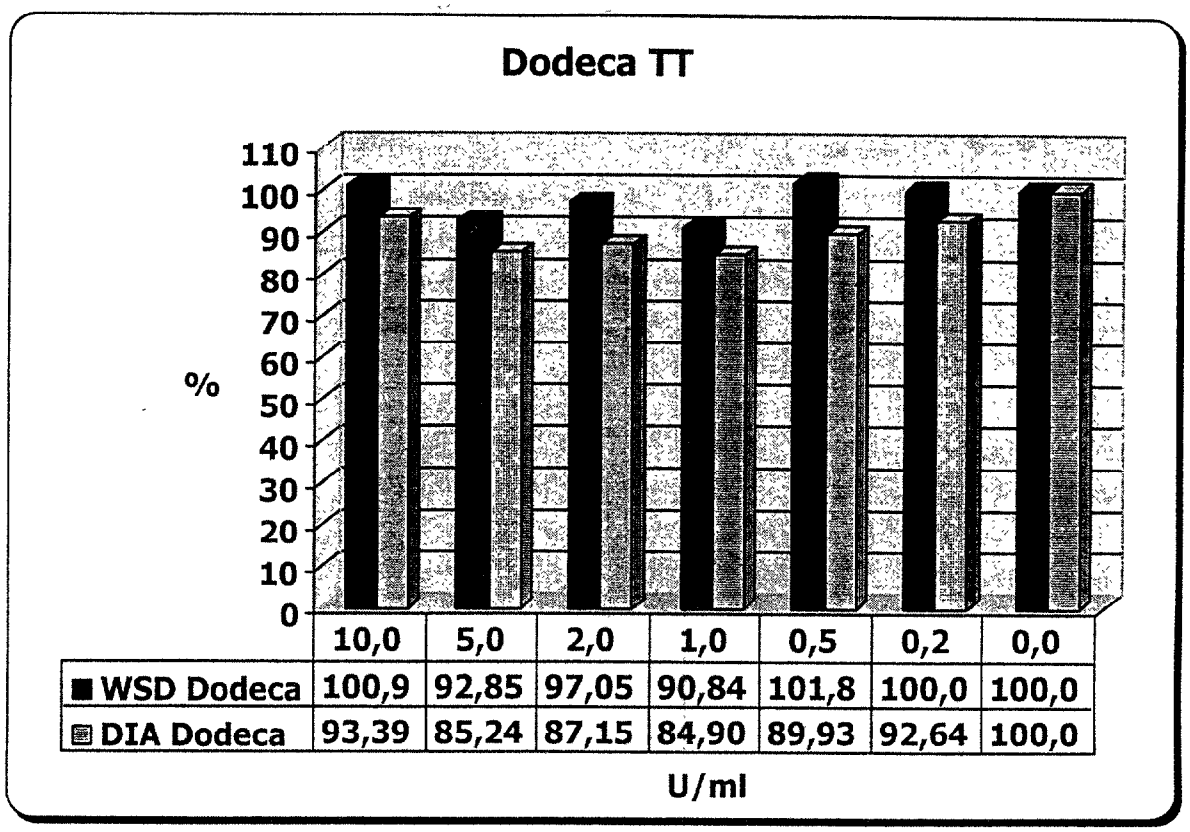

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| WSD Dodeca | 85,87 | 96,42 | 103,8 | 106,7 | 105,7 | 104,6 | 100,0 |
| DIA Dodeca | 84,69 | 97,34 | 100,8 | 104,8 | 109,2 | 104,8 | 100,0 |




| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| WSD Dodeca | 309,2 | 254,5 | 201,1 | 178,2 | 157,9 | 147,2 | 100,0 |
| DIA Dodeca | 299,8 | 273,5 | 243,0 | 191,2 | 171,8 | 124,3 | 100,0 |

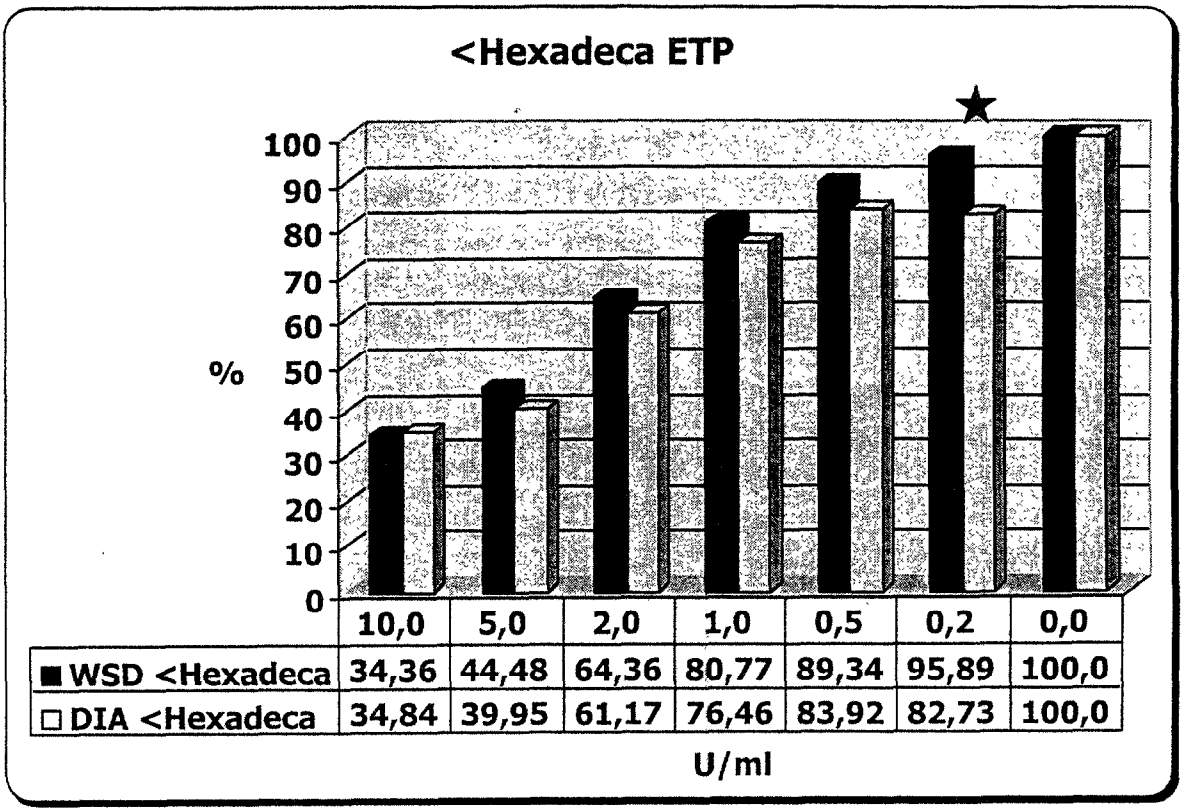



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD Dodeca | 282,0 | 155,1 | 135,4 | 108,8 | 107,3 | 105,6 | 100,0 |
| ▤ DIA Dodeca | 284,3 | 183,0 | 130,6 | 112,7 | 109,0 | 101,6 | 100,0 |

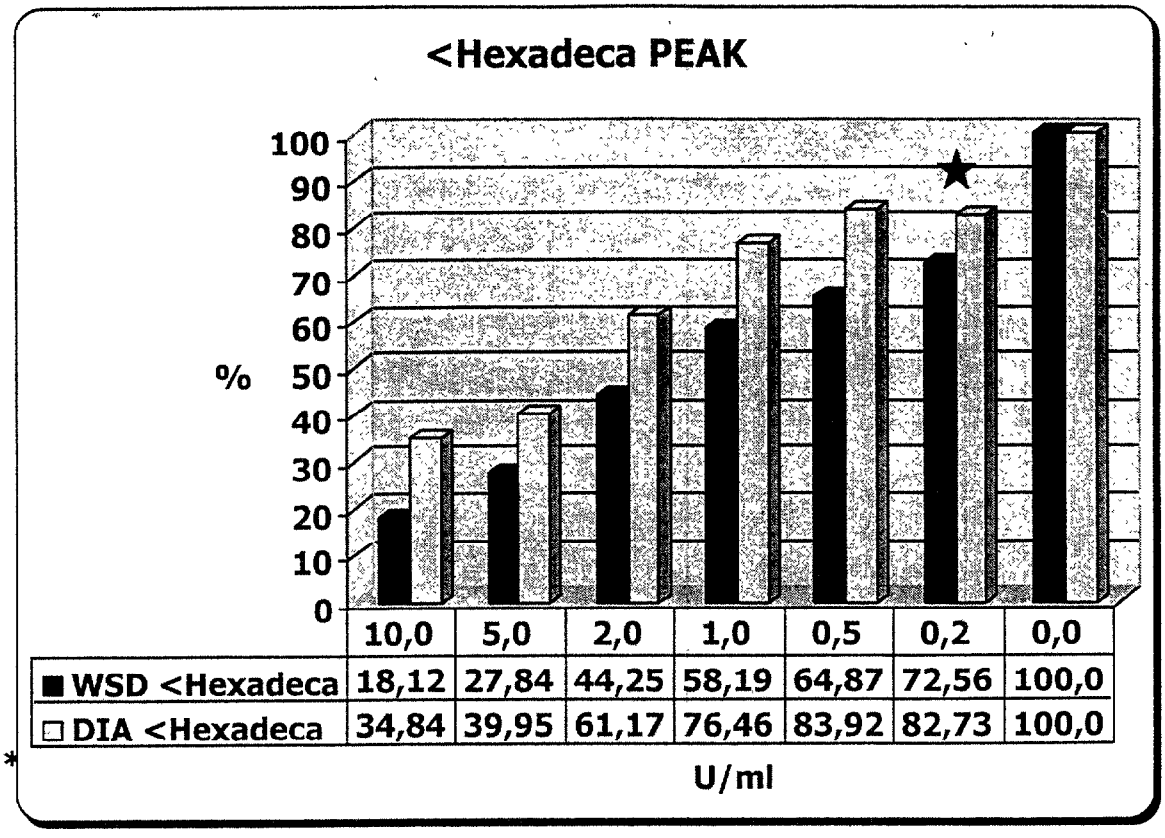



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| WSD Dodeca | 100,9 | 92,85 | 97,05 | 90,84 | 101,8 | 100,0 | 100,0 |
| DIA Dodeca | 93,39 | 85,24 | 87,15 | 84,90 | 89,93 | 92,64 | 100,0 |

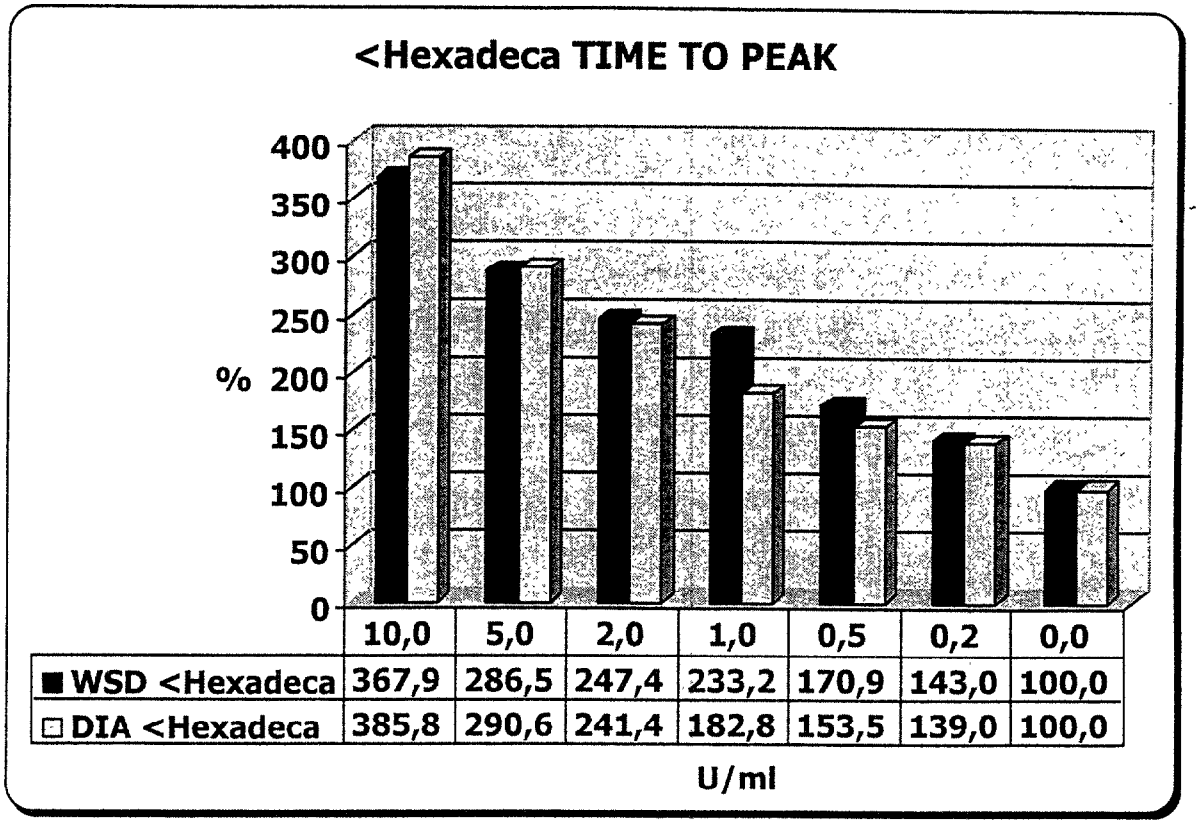
e) < Hexasaccharide

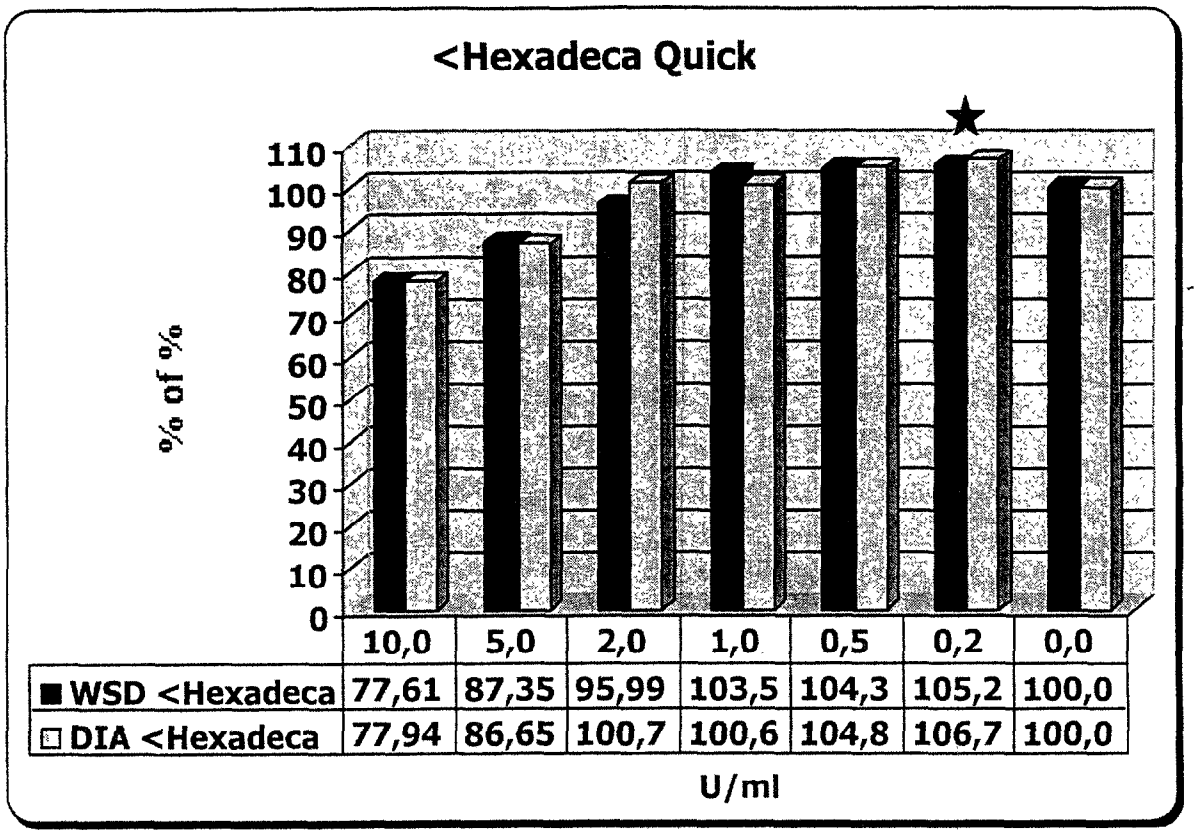



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD <Hexadeca | 34,36 | 44,48 | 64,36 | 80,77 | 89,34 | 95,89 | 100,0 |
| □ DIA <Hexadeca | 34,84 | 39,95 | 61,17 | 76,46 | 83,92 | 82,73 | 100,0 |




| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD <Hexadeca | 18,12 | 27,84 | 44,25 | 58,19 | 64,87 | 72,56 | 100,0 |
| □ DIA <Hexadeca | 34,84 | 39,95 | 61,17 | 76,46 | 83,92 | 82,73 | 100,0 |

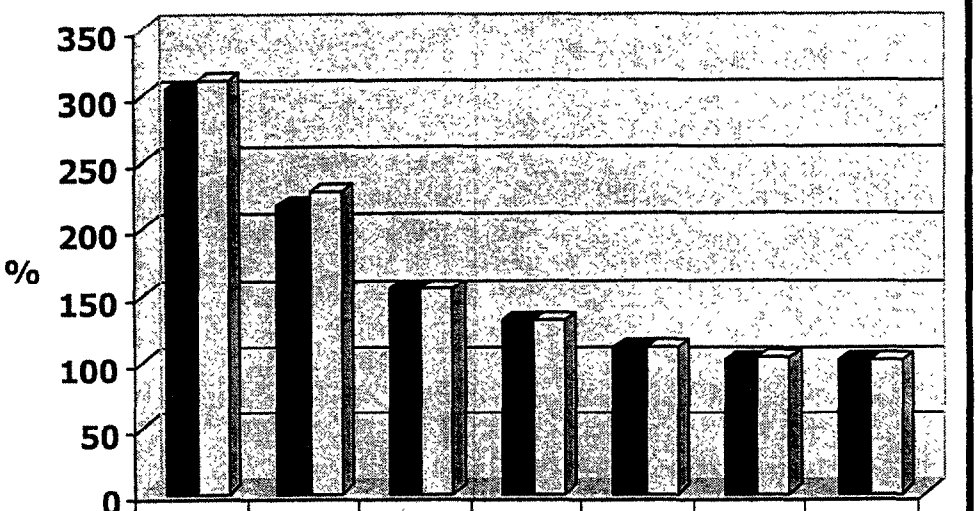

<Hexadeca TIME TO PEAK
400
350
300
250
% 200
150
100
50
0
10,0 5,0 2,0 1,0 0,5 0,2 0,0
WSD <Hexadeca 367,9 286,5 247,4 233,2 170,9 143,0 100,0
DIA <Hexadeca 385,8 290,6 241,4 182,8 153,5 139,0 100,0
U/ml




| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD <Hexadeca | 77,61 | 87,35 | 95,99 | 103,5 | 104,3 | 105,2 | 100,0 |
| □ DIA <Hexadeca | 77,94 | 86,65 | 100,7 | 100,6 | 104,8 | 106,7 | 100,0 |

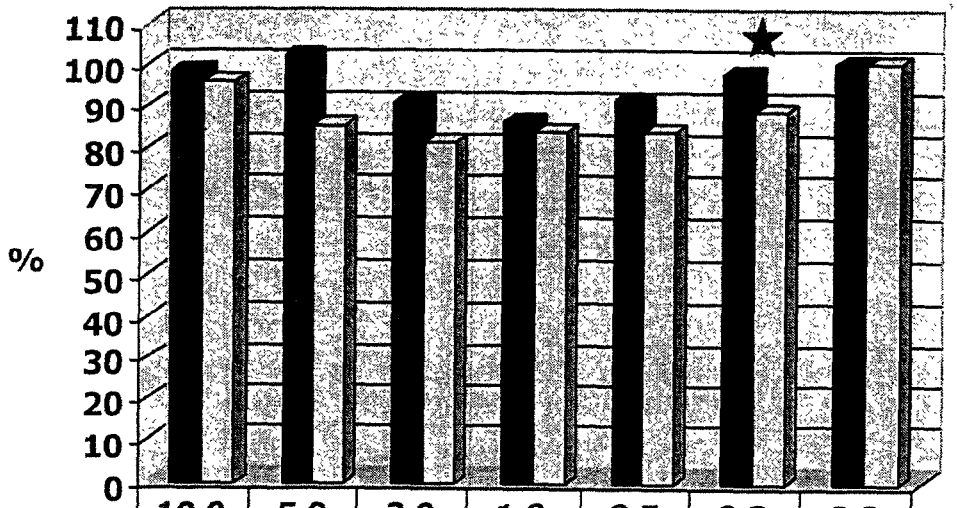
* = $p < 0,05$

## <Hexadeca aPTT




| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD <Hexadeca | 304,3 | 217,6 | 154,4 | 129,9 | 110,7 | 99,87 | 100,0 |
| □ DIA <Hexadeca | 311,3 | 227,1 | 154,1 | 130,0 | 110,0 | 102,7 | 100,0 |

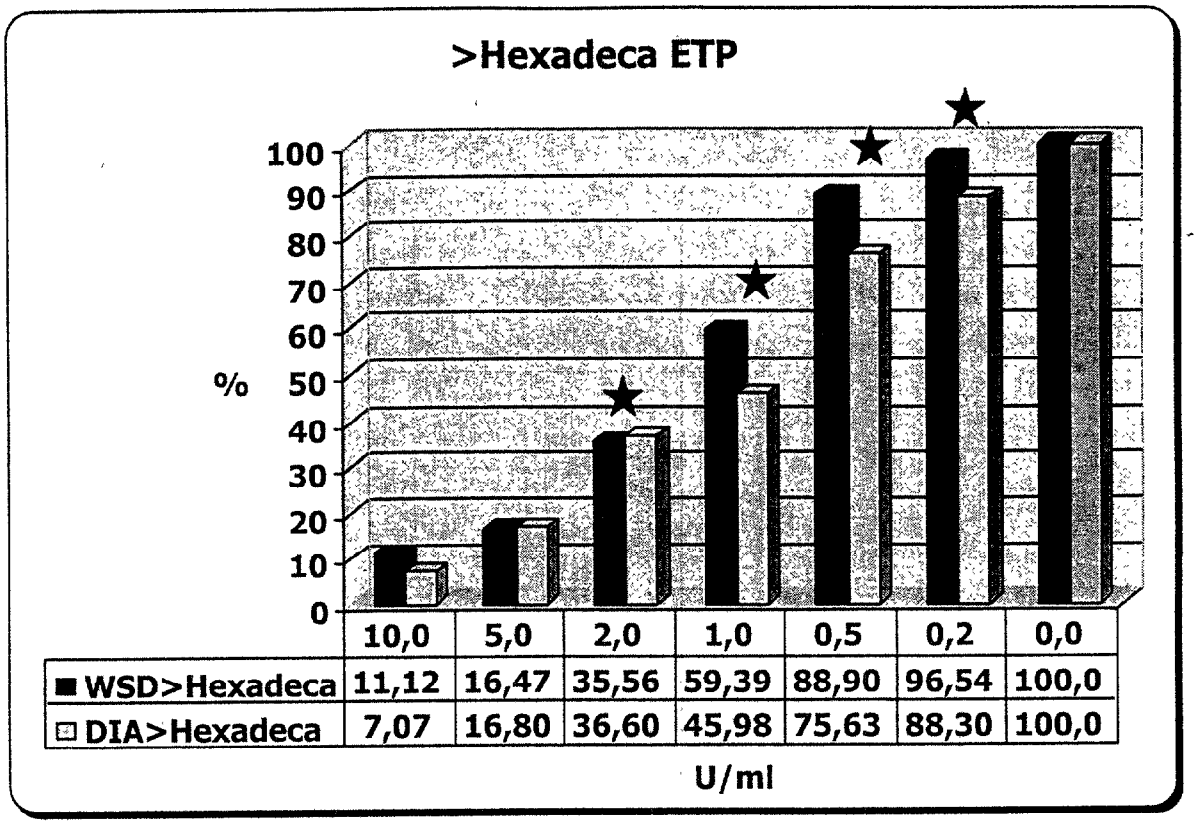
U/ml




| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD <Hexadeca | 98,12 | 101,8 | 90,46 | 86,36 | 91,27 | 97,86 | 100,0 |
| □ DIA <Hexadeca | 95,70 | 85,34 | 81,24 | 83,47 | 83,70 | 88,97 | 100,0 |

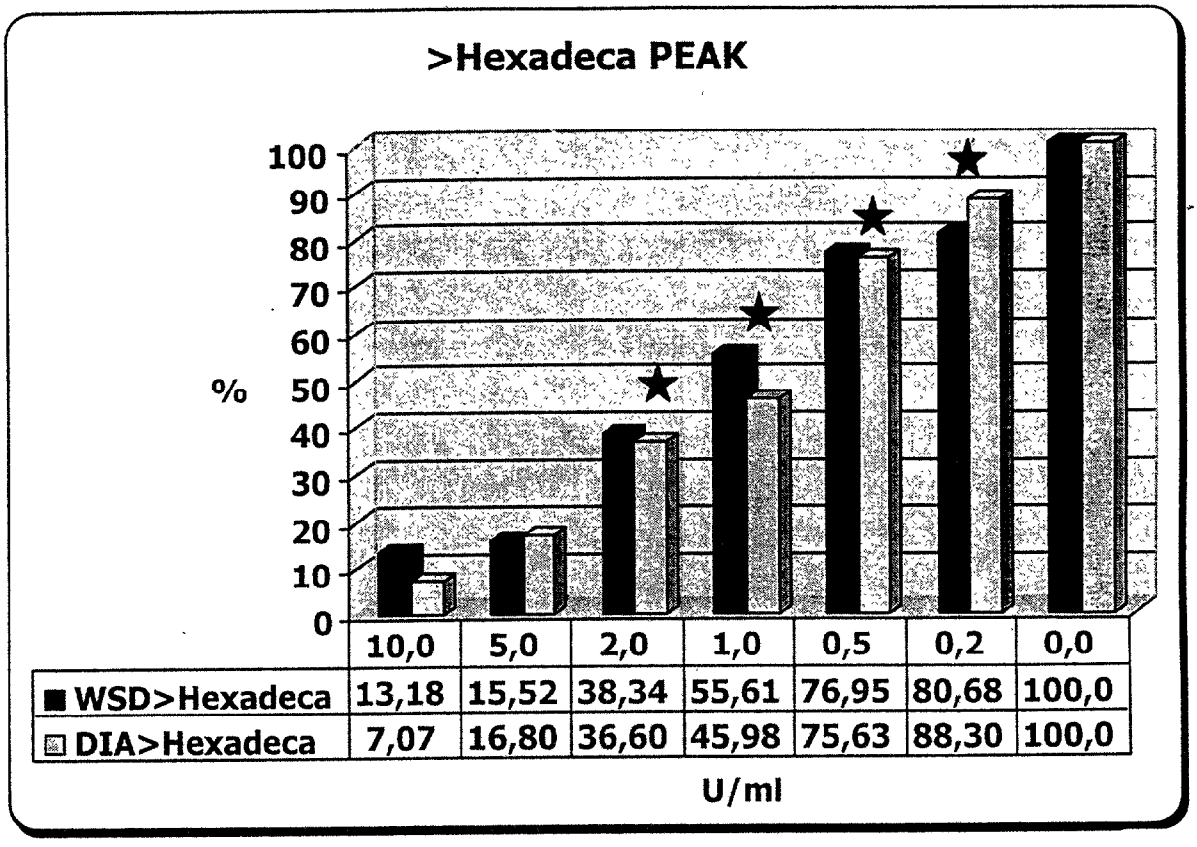
U/ml

f) > Hexasaccharide:



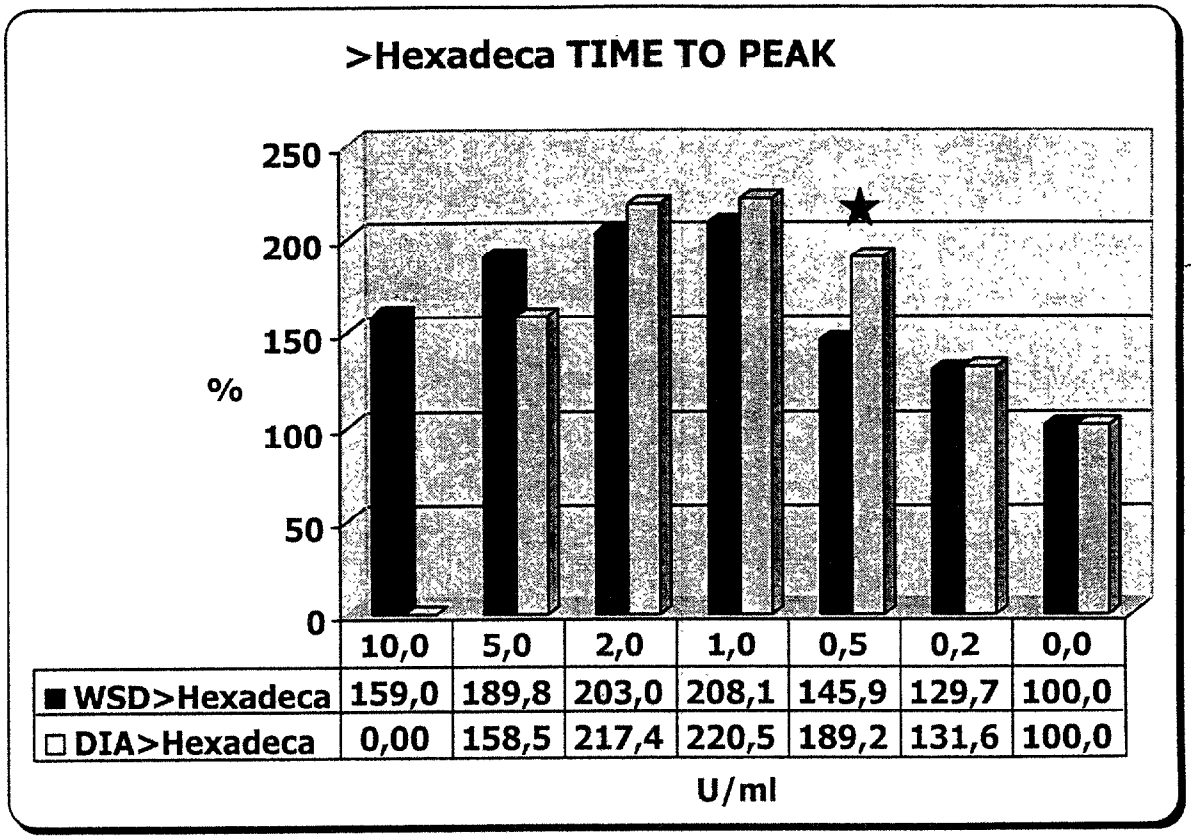

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD>Hexadeca | 11,12 | 16,47 | 35,56 | 59,39 | 88,90 | 96,54 | 100,0 |
| □ DIA>Hexadeca | 7,07 | 16,80 | 36,60 | 45,98 | 75,63 | 88,30 | 100,0 |

* = $p < 0,05$



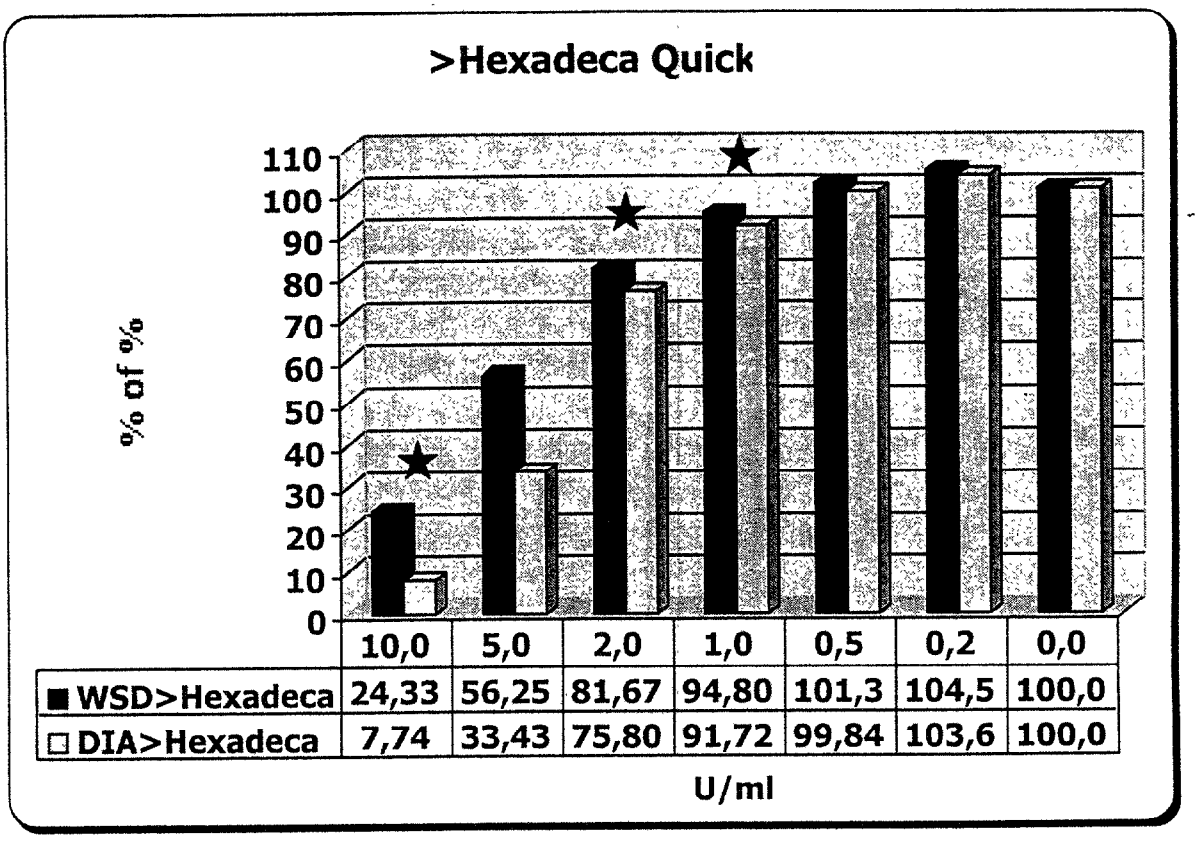

| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD>Hexadeca | 13,18 | 15,52 | 38,34 | 55,61 | 76,95 | 80,68 | 100,0 |
| ▨ DIA>Hexadeca | 7,07 | 16,80 | 36,60 | 45,98 | 75,63 | 88,30 | 100,0 |

* = $p < 0,05$

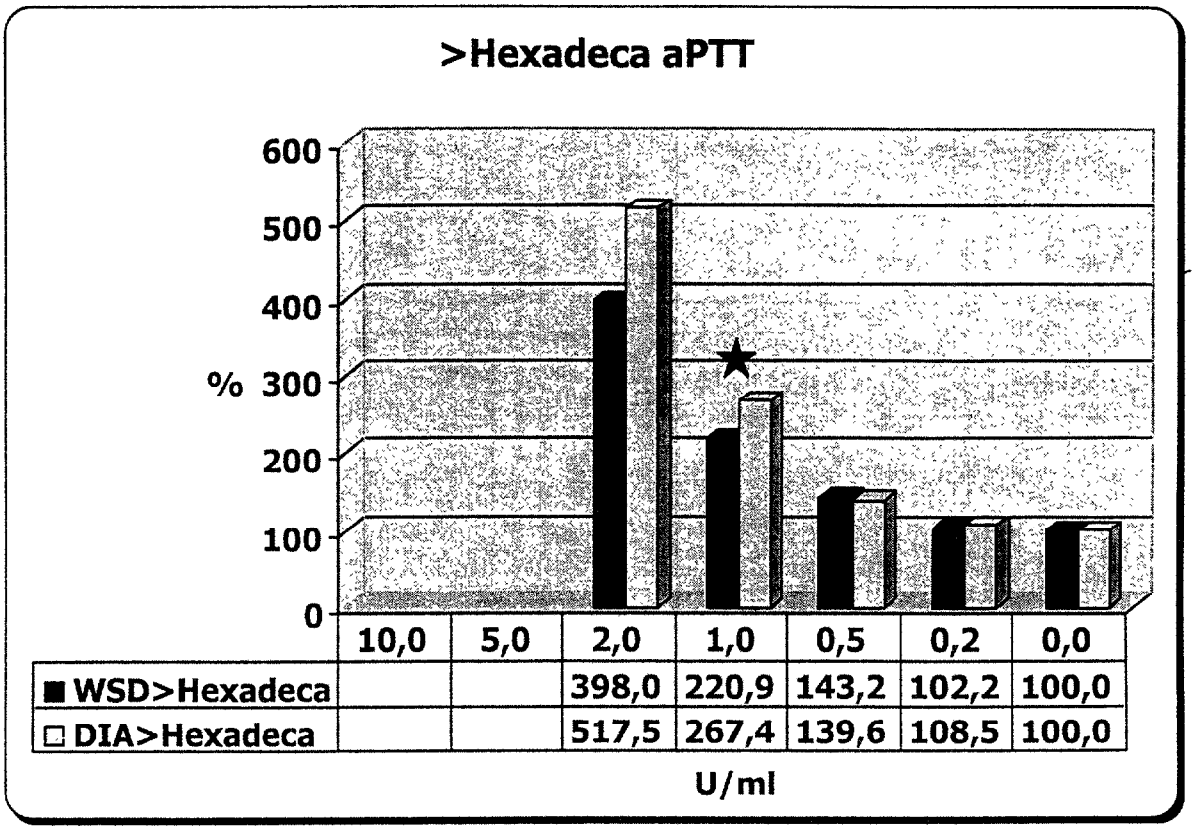



| | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| ■ WSD>Hexadeca | 159,0 | 189,8 | 203,0 | 208,1 | 145,9 | 129,7 | 100,0 |
| □ DIA>Hexadeca | 0,00 | 158,5 | 217,4 | 220,5 | 189,2 | 131,6 | 100,0 |

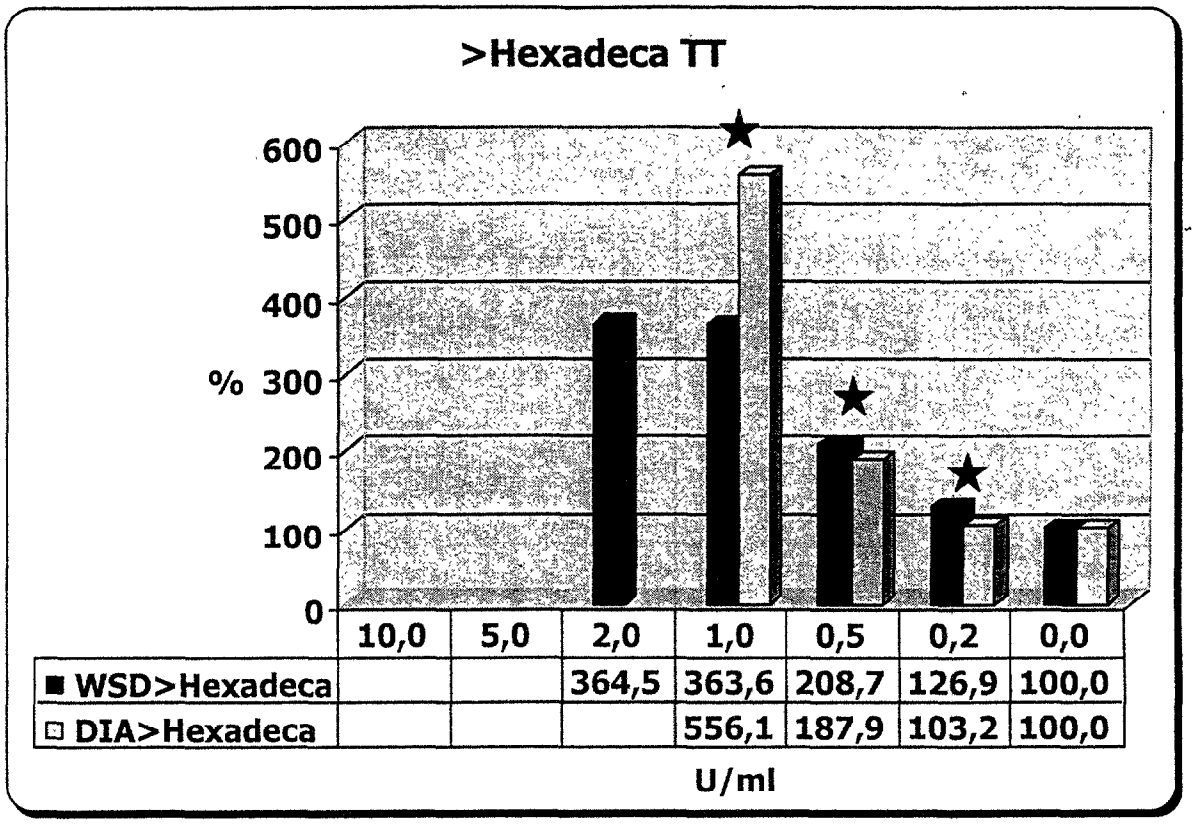
* = $p < 0,05$


>Hexadeca Quick
% of %
110
100
90
80
70
60
50
40
30
20
10
0
10,0 5,0 2,0 1,0 0,5 0,2 0,0
WSD>Hexadeca 24,33 56,25 81,67 94,80 101,3 104,5 100,0
DIA>Hexadeca 7,74 33,43 75,80 91,72 99,84 103,6 100,0
U/ml

* = p < 0,05


>Hexadeca aPTT
600
500
400
% 300
200
100
0
10,0 5,0 2,0 1,0 0,5 0,2 0,0
WSD>Hexadeca 398,0 220,9 143,2 102,2 100,0
DIA>Hexadeca 517,5 267,4 139,6 108,5 100,0
U/ml

* = $p < 0,05$


>Hexadeca TT
600
500
400
% 300
200
100
0
10,0 5,0 2,0 1,0 0,5 0,2 0,0
WSD>Hexadeca 364,5 363,6 208,7 126,9 100,0
DIA>Hexadeca 556,1 187,9 103,2 100,0
U/ml

* = $p < 0,05$

Summary (statistically significant changes, $p<0.05$):

*Hexasaccharides*

- All WSD concentrations except for 2 U/ml cause a stronger reduction in ETP, and, except for 0.2 and 2 U/ml, in PEAK, too
- WSD reduced Quick assay values at 5 and 2 U/ml stronger than DIA
- WSD influence on TT was larger at 10 (increase) and 5 U/ml (reduction) DIA was more effective in TT reduction at 1 and 0.5 U/ml

- No significant differences in Time to PEAK and aPTT

*Octasaccharides*

- DIA caused a larger increase in the Quick assay value at 0.2 U/ml

- No further significant differences in test parameters for OCTA WSD/DIA pair

*Decasaccharides*

- While DIA reduced ETP more at 0.2 U/ml
- WSD was more effective at 0.2 U/ml in decreasing PEAK
- DIA increased Quick values at 0.2 U/ml, but WSD reduced them at 2U/ml.
- WSD prolonged aPTT at 10 U/ml more effective than DIA
- DIA caused a larger TT reduction at 0.2 U/ml

- almost all effects at 0.2 U/ml (lowest concentration)

*Dodecasaccharides*

- No significant differences for WSD and DIA Dodeca

*<Hexadecasaccharides*

- DIA was more effective at 0.2 U/ml in ETP reduction
- WSD caused a stronger reduction at 0.2 U/ml in PEAK
- DIA generated a larger but minimal increase at 0.2 U/ml in the Quick assay
- TT was reduced stronglier by DIA at 0.2 U/ml

*>Hexadecasaccharides*

- DIA reduced the ETP more at 0.5, 0.2 U/ml, WSD at 2 U/ml
- DIA was more effective in PEAK reduction at 2, 1, 0.5 U/ml, WSD stronger at 0.2 U/ml
- WSD prolonged time to PEAK at 0.5 U/ml stronger than DIA.
- DIA reduced Quick value more than WSD at 10, 2 and 1 U/ml

- The aPTT was stronglier prolonged by DIA at 1 U/ml
- DIA generated a larger increase in TT at 1U/ml, whereas WSD was more effective than DIA at lower concentrations (0.5 and 0.2 U/ml)

5. Data tables: Heparinoid Reagents' Dose Response, MEAN (rows with numbers); SD (below MEAN); in %

**5.1 ETP:**

| ETP | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 | | |
|---|---|---|---|---|---|---|---|---|---|
| **WSD Hexa** | 83,86 | 142,43 | 225,81 | 345,00 | 477,00 | 594,31 | 667,49 | | MW |
| | 20,81 | 31,41 | 105,41 | 104,97 | 50,33 | 105,91 | 91,88 | | STABW |
| **WSD Octa** | 195,14 | 263,64 | 352,65 | 496,08 | 557,77 | 593,57 | 667,49 | | MW |
| | 46,96 | 86,59 | 134,31 | 60,55 | 95,08 | 114,48 | 91,88 | | STABW |
| **WSD Deca** | 248,75 | 352,87 | 467,82 | 533,79 | 562,39 | 652,50 | 667,49 | | MW |
| | 62,06 | 90,75 | 100,73 | 69,14 | 74,73 | 123,39 | 91,88 | | STABW |
| **WSD Dodeca** | 269,47 | 292,33 | 470,02 | 556,16 | 604,29 | 653,61 | 667,49 | | MW |
| | 64,04 | 140,54 | 96,16 | 74,83 | 94,99 | 96,65 | 91,88 | | STABW |
| **WSD <Hexadeca** | 216,50 | 282,44 | 419,23 | 529,71 | 592,79 | 638,98 | 667,49 | | MW |
| | 54,20 | 140,27 | 98,44 | 80,46 | 81,87 | 94,30 | 91,88 | | STABW |
| **WSD>Hexadeca** | 71,37 | 104,29 | 272,90 | 387,18 | 593,36 | 643,46 | 667,49 | | MW |
| | 51,33 | 50,68 | 56,81 | 115,37 | 85,96 | 92,13 | 91,88 | | STABW |
| **DIA Hexa** | 94,77 | 192,91 | 270,40 | 433,38 | 526,69 | 615,09 | 667,49 | | MW |
| | 19,95 | 41,80 | 108,59 | 111,43 | 62,27 | 66,02 | 91,88 | | STABW |
| **DIA Octa** | 200,90 | 280,81 | 378,17 | 512,96 | 546,59 | 600,21 | 667,49 | | MW |
| | 49,37 | 79,00 | 95,92 | 86,34 | 59,88 | 113,08 | 91,88 | | STABW |
| **DIA Deca** | 213,30 | 286,87 | 451,61 | 530,44 | 591,34 | 624,87 | 667,49 | | MW |
| | 110,70 | 134,30 | 106,05 | 60,20 | 64,13 | 115,06 | 91,88 | | STABW |
| **DIA Dodeca** | 218,14 | 281,02 | 435,88 | 529,47 | 594,76 | 624,35 | 667,49 | | MW |
| | 112,30 | 114,19 | 84,06 | 53,72 | 89,29 | 119,72 | 91,88 | | STABW |
| **DIA <Hexadeca** | 218,54 | 251,50 | 397,82 | 502,28 | 558,55 | 562,50 | 667,49 | | MW |
| | 39,41 | 122,18 | 82,83 | 44,08 | 74,92 | 173,12 | 91,88 | | STABW |
| **DIA>Hexadeca** | 45,86 | 106,49 | 233,12 | 296,53 | 504,28 | 591,29 | 667,49 | | MW |
| | 12,39 | 51,89 | 53,49 | 135,75 | 71,61 | 112,86 | 91,88 | | STABW |

**5.2 PEAK:**

| PEAK | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | | |
|---|---|---|---|---|---|---|---|---|---|
| **WSD Hexa** | 2,57 | 3,92 | 6,77 | 12,42 | 16,89 | 21,85 | 34,19 | | MW |
| | 0,42 | 0,54 | 2,24 | 3,17 | 4,08 | 7,34 | 5,60 | | STABW |
| **WSD Octa** | 5,00 | 7,31 | 12,83 | 16,31 | 21,28 | 21,70 | 34,19 | | MW |
| | 1,20 | 2,40 | 3,39 | 3,41 | 6,94 | 4,39 | 5,60 | | STABW |
| **WSD Deca** | 6,63 | 10,93 | 15,93 | 19,29 | 20,35 | 25,29 | 34,19 | | MW |
| | 1,85 | 3,86 | 4,64 | 5,08 | 5,08 | 7,47 | 5,60 | | STABW |
| **WSD Dodeca** | 7,70 | 9,12 | 15,92 | 19,71 | 22,94 | 25,78 | 34,19 | | MW |
| | 2,35 | 4,10 | 4,28 | 4,84 | 5,60 | 6,54 | 5,60 | | STABW |
| **WSD <Hexadeca** | 5,70 | 8,74 | 14,44 | 19,05 | 21,62 | 24,25 | 34,19 | | MW |
| | 1,40 | 3,90 | 4,92 | 4,36 | 4,46 | 5,30 | 5,60 | | STABW |
| **WSD>Hexadeca** | 3,55 | 4,76 | 12,18 | 18,49 | 25,98 | 27,01 | 34,19 | | MW |
| | 1,55 | 2,32 | 4,04 | 5,56 | 9,65 | 7,89 | 5,60 | | STABW |
| **DIA Hexa** | 3,30 | 5,53 | 8,42 | 16,20 | 18,99 | 22,54 | 34,19 | | MW |
| | 0,55 | 0,79 | 1,61 | 4,60 | 3,84 | 4,68 | 5,60 | | STABW |
| **DIA Octa** | 5,42 | 7,89 | 12,94 | 17,84 | 19,45 | 21,76 | 34,19 | | MW |
| | 1,32 | 2,52 | 4,12 | 6,15 | 4,96 | 6,05 | 5,60 | | STABW |
| **DIA Deca** | 6,55 | 8,25 | 16,20 | 17,97 | 21,47 | 23,50 | 34,19 | | MW |
| | 1,70 | 3,27 | 4,92 | 4,04 | 4,71 | 6,44 | 5,60 | | STABW |
| **DIA Dodeca** | 7,09 | 9,31 | 14,44 | 18,14 | 22,45 | 24,23 | 34,19 | | MW |
| | 2,02 | 2,46 | 3,62 | 3,70 | 4,74 | 6,98 | 5,60 | | STABW |
| **DIA <Hexadeca** | 5,72 | 7,96 | 13,44 | 17,02 | 19,36 | 20,80 | 34,19 | | MW |
| | 1,19 | 2,79 | 4,49 | 3,89 | 4,39 | 6,71 | 5,60 | | STABW |
| **DIA>Hexadeca** | 2,61 | 5,36 | 10,55 | 13,39 | 21,59 | 24,71 | 34,19 | | MW |
| | #DIV/0! | 2,47 | 4,04 | 6,48 | 7,33 | 8,42 | 5,60 | | STABW |

Empty boxes in SD rows indicate a number of values below two, thus no SD could be calculated. Empty boxes in MEAN rows indicate no thrombin or fibrin formation detectable in all volunteers.

## 5.3 TIME TO PEAK:

| TIMETOPEAK | 10,0 | 5,0 | 2,0 | 1,0 | 0,5 | 0,2 | 0,0 |
|---|---|---|---|---|---|---|---|
| **WSD Hexa** | 51,27 | 49,27 | 42,06 | 36,50 | 28,67 | 26,00 | 14,33 |
| | 13,85 | 15,05 | 16,86 | 19,07 | 12,76 | 8,90 | 4,45 |
| **WSD Octa** | 45,93 | 33,93 | 36,94 | 28,06 | 22,89 | 21,53 | 14,33 |
| | 11,89 | 17,20 | 18,49 | 11,83 | 7,09 | 5,63 | 4,45 |
| **WSD Deca** | 43,33 | 34,07 | 32,33 | 30,06 | 24,89 | 20,50 | 14,33 |
| | 18,41 | 14,80 | 13,83 | 9,77 | 10,01 | 4,75 | 4,45 |
| **WSD Dodeca** | 34,13 | 36,44 | 29,61 | 25,33 | 21,89 | 19,56 | 14,33 |
| | 18,56 | 18,38 | 16,25 | 11,70 | 7,80 | 5,66 | 4,45 |
| **WSD <Hexadeca** | 32,60 | 39,06 | 34,56 | 31,06 | 22,78 | 20,06 | 14,33 |
| | 19,07 | 16,77 | 14,13 | 8,98 | 5,12 | 6,18 | 4,45 |
| **WSD>Hexadeca** | 24,00 | 22,97 | 24,73 | 29,47 | 21,33 | 17,97 | 14,33 |
| | 0,94 | 8,58 | 9,20 | 18,98 | 13,20 | 8,07 | 4,45 |
| **DIA Hexa** | 56,73 | 51,33 | 41,56 | 35,61 | 28,56 | 21,50 | 14,33 |
| | 7,88 | 8,62 | 15,01 | 16,14 | 9,79 | 6,04 | 4,45 |
| **DIA Octa** | 49,13 | 40,33 | 34,33 | 27,67 | 24,83 | 20,56 | 14,33 |
| | 8,88 | 13,82 | 15,09 | 11,39 | 10,50 | 4,19 | 4,45 |
| **DIA Deca** | 44,60 | 40,78 | 31,17 | 26,56 | 22,94 | 19,83 | 14,33 |
| | 11,61 | 15,82 | 17,68 | 12,58 | 9,41 | 4,44 | 4,45 |
| **DIA Dodeca** | 37,87 | 38,56 | 34,11 | 28,00 | 23,33 | 17,78 | 14,33 |
| | 18,94 | 19,18 | 16,20 | 14,04 | 5,88 | 6,47 | 4,45 |
| **DIA <Hexadeca** | 42,00 | 40,44 | 33,06 | 25,28 | 21,22 | 19,61 | 14,33 |
| | 20,87 | 15,31 | 13,03 | 9,63 | 6,55 | 7,40 | 4,45 |
| **DIA>Hexadeca** | 27,67 | 23,37 | 27,20 | 31,64 | 27,25 | 18,22 | 14,33 |
| | | 6,61 | 6,55 | 17,55 | 13,43 | 7,21 | 4,45 |

Empty boxes in SD rows indicate a number of values below two, thus no SD could be calculated. Empty boxes in MEAN rows indicate no thrombin or fibrin formation detectable in all volunteers.

## 5.4 Quick (%):

| Quick | 10,00 | 5,00 | 2,50 | 1,00 | 0,50 | 0,20 | 0,00 | | |
|---|---|---|---|---|---|---|---|---|---|
| WSD Hexa | 44,17 | 55,33 | 67,33 | 76,50 | 79,50 | 84,00 | 85,17 | | MW |
| | 10,65 | 7,66 | 7,89 | 6,19 | 8,02 | 6,48 | 6,40 | | STABW |
| WSD Octa | 64,50 | 70,33 | 78,83 | 83,33 | 85,83 | 87,33 | 85,17 | | MW |
| | 8,31 | 8,45 | 7,99 | 6,62 | 7,19 | 5,50 | 6,40 | | STABW |
| WSD Deca | 69,33 | 79,40 | 81,00 | 85,00 | 86,83 | 87,33 | 85,17 | | MW |
| | 8,38 | 6,73 | 11,71 | 10,66 | 8,23 | 6,62 | 6,40 | | STABW |
| WSD Dodeca | 73,33 | 82,17 | 88,33 | 90,83 | 90,17 | 89,17 | 85,17 | | MW |
| | 9,83 | 7,31 | 5,54 | 6,24 | 8,91 | 6,94 | 6,40 | | STABW |
| WSD <Hexadeca | 66,17 | 74,50 | 81,83 | 88,17 | 88,83 | 89,67 | 85,17 | | MW |
| | 7,63 | 7,58 | 7,63 | 6,55 | 7,05 | 7,03 | 6,40 | | STABW |
| WSD>Hexadeca | 21,20 | 48,20 | 69,67 | 80,83 | 86,33 | 89,00 | 85,17 | | MW |
| | 9,26 | 3,56 | 9,18 | 8,64 | 7,63 | 7,24 | 6,40 | | STABW |
| DIA Hexa | 49,17 | 59,67 | 72,67 | 77,83 | 82,67 | 87,17 | 87,33 | | MW |
| | 5,56 | 8,36 | 8,33 | 7,25 | 6,98 | 6,91 | 6,35 | | STABW |
| DIA Octa | 67,00 | 71,50 | 80,67 | 84,50 | 88,17 | 90,50 | 87,33 | | MW |
| | 6,07 | 7,77 | 6,31 | 6,12 | 6,08 | 5,99 | 6,35 | | STABW |
| DIA Deca | 71,17 | 76,83 | 87,67 | 87,50 | 90,67 | 90,33 | 87,33 | | MW |
| | 7,14 | 7,96 | 4,68 | 7,71 | 5,50 | 6,62 | 6,35 | | STABW |
| DIA Dodeca | 72,17 | 82,67 | 86,00 | 89,33 | 92,83 | 89,33 | 87,33 | | MW |
| | 7,03 | 6,02 | 9,12 | 7,99 | 3,66 | 7,03 | 6,35 | | STABW |
| DIA <Hexadeca | 66,50 | 73,83 | 85,67 | 85,67 | 89,17 | 90,83 | 87,33 | | MW |
| | 7,45 | 8,13 | 5,75 | 7,15 | 4,96 | 5,60 | 6,35 | | STABW |
| DIA>Hexadeca | 7,00 | 29,00 | 64,67 | 78,17 | 85,17 | 88,17 | 87,33 | | MW |
| | 1,41 | 8,37 | 8,09 | 8,54 | 9,72 | 5,53 | 6,35 | | STABW |

**5.5 PTT (s):**

| PTT | 10,00 | 5,00 | 2,50 | 1,00 | 0,50 | 0,20 | 0,00 | | |
|---|---|---|---|---|---|---|---|---|---|
| **WSD Hexa** | | 189,85 | 79,13 | 53,16 | 41,65 | 41,04 | 36,72 | | MW |
| | | 162,85 | 23,45 | 25,81 | 10,97 | 7,53 | 3,13 | | STABW |
| **WSD Octa** | 159,15 | 75,58 | 49,90 | 44,65 | 42,87 | 39,76 | 36,72 | | MW |
| | 51,69 | 26,73 | 11,57 | 6,47 | 6,08 | 6,48 | 3,13 | | STABW |
| **WSD Deca** | 111,42 | 69,93 | 62,68 | 43,00 | 41,02 | 36,30 | 36,72 | | MW |
| | 33,81 | 19,60 | 20,60 | 17,88 | 12,74 | 8,50 | 3,13 | | STABW |
| **WSD Dodeca** | 103,06 | 57,98 | 49,78 | 39,97 | 39,43 | 40,20 | 36,72 | | MW |
| | 20,01 | 27,30 | 13,80 | 6,47 | 5,27 | 4,49 | 3,13 | | STABW |
| **WSD <Hexadeca** | 111,46 | 80,03 | 56,78 | 49,10 | 40,76 | 36,20 | 36,72 | | MW |
| | 35,80 | 12,52 | 8,06 | 8,63 | 10,13 | 6,70 | 3,13 | | STABW |
| **WSD>Hexadeca** | | | 152,45 | 81,58 | 52,57 | 37,38 | 36,72 | | MW |
| | | | 83,46 | 20,62 | 7,64 | 5,15 | 3,13 | | STABW |
| **DIA Hexa** | | 191,60 | 85,40 | 52,88 | 45,44 | 40,40 | 36,50 | | MW |
| | | 98,99 | 22,85 | 22,58 | 10,08 | 5,80 | 2,88 | | STABW |
| **DIA Octa** | 133,97 | 74,18 | 49,92 | 48,08 | 43,38 | 38,60 | 36,50 | | MW |
| | 29,33 | 25,30 | 11,33 | 7,07 | 5,94 | 7,00 | 2,88 | | STABW |
| **DIA Deca** | 100,58 | 78,65 | 55,08 | 46,80 | 37,80 | 35,72 | 36,50 | | MW |
| | 21,60 | 15,58 | 8,71 | 10,62 | 8,18 | 4,12 | 2,88 | | STABW |
| **DIA Dodeca** | 103,66 | 68,54 | 47,76 | 41,60 | 40,08 | 38,42 | 36,50 | | MW |
| | 17,41 | 27,24 | 9,81 | 9,73 | 5,55 | 5,20 | 2,88 | | STABW |
| **DIA <Hexadeca** | 113,74 | 83,53 | 56,67 | 47,92 | 40,06 | 37,67 | 36,50 | | MW |
| | 30,07 | 15,88 | 8,61 | 8,42 | 5,27 | 3,33 | 2,88 | | STABW |
| **DIA>Hexadeca** | | | 207,15 | 99,05 | 50,58 | 39,93 | 36,50 | | MW |
| | | | 124,95 | 27,67 | 1,05 | 5,21 | 2,88 | | STABW |

Empty boxes in SD rows indicate a number of values below two, thus no SD could be calculated. Empty boxes in MEAN rows indicate no thrombin or fibrin formation detectable in all volunteers.

**5.6 TT %:**

| TT (s) | 20,00 | 5,00 | 2,00 | 1,00 | 0,50 | 0,25 | 0,00 | | |
|---|---|---|---|---|---|---|---|---|---|
| **WSD Hexa** | 24,28 | 18,15 | 17,12 | 16,65 | 18,82 | 17,58 | 19,77 | | MW |
| | 10,33 | 2,48 | 3,79 | 2,38 | 7,22 | 2,39 | 1,69 | | STABW |
| **WSD Octa** | 17,47 | 16,32 | 17,22 | 19,68 | 17,25 | 22,33 | 19,77 | | MW |
| | 6,03 | 1,37 | 4,21 | 9,44 | 3,94 | 11,48 | 1,69 | | STABW |
| **WSD Deca** | 22,47 | 16,58 | 18,10 | 19,20 | 18,88 | 19,58 | 19,77 | | MW |
| | 12,49 | 0,95 | 5,72 | 8,27 | 5,19 | 3,49 | 1,69 | | STABW |
| **WSD Dodeca** | 20,27 | 18,55 | 19,53 | 18,03 | 20,40 | 19,87 | 19,77 | | MW |
| | 8,61 | 5,90 | 8,76 | 3,63 | 7,60 | 4,46 | 1,69 | | STABW |
| **WSD <Hexadeca** | 19,50 | 20,58 | 18,03 | 16,56 | 18,12 | 19,35 | 19,77 | | MW |
| | 4,55 | 12,00 | 4,92 | 0,68 | 3,80 | 2,99 | 1,69 | | STABW |
| **WSD>Hexadeca** | | | 67,80 | 72,66 | 41,53 | 25,02 | 19,77 | | MW |
| | | | | 34,45 | 13,04 | 7,37 | 1,69 | | STABW |
| **DIA Hexa** | 20,27 | 19,98 | 16,48 | 15,60 | 15,70 | 17,62 | 18,38 | | MW |
| | 3,73 | 6,33 | 2,24 | 1,63 | 1,41 | 3,52 | 2,30 | | STABW |
| **DIA Octa** | 18,55 | 17,32 | 17,05 | 16,50 | 17,07 | 17,28 | 18,38 | | MW |
| | 4,13 | 3,09 | 4,04 | 2,34 | 2,18 | 2,27 | 2,30 | | STABW |
| **DIA Deca** | 17,47 | 17,15 | 16,70 | 16,87 | 17,58 | 17,40 | 18,38 | | MW |
| | 2,11 | 3,32 | 2,22 | 2,68 | 2,43 | 2,38 | 2,30 | | STABW |
| **DIA Dodeca** | 18,48 | 16,34 | 17,27 | 16,78 | 17,77 | 18,25 | 18,38 | | MW |
| | 2,53 | 0,79 | 2,68 | 1,91 | 1,80 | 1,91 | 2,30 | | STABW |
| **DIA <Hexadeca** | 19,03 | 16,85 | 15,56 | 16,47 | 16,48 | 17,52 | 18,38 | | MW |
| | 4,28 | 1,59 | 0,47 | 1,70 | 1,13 | 1,30 | 2,30 | | STABW |
| **DIA>Hexadeca** | | | | 106,85 | 37,47 | 20,35 | 18,38 | | MW |
| | | | | 17,32 | 10,27 | 0,97 | 2,30 | | STABW |

Empty boxes in SD rows indicate a number of values below two, thus no SD could be calculated. Empty boxes in MEAN rows indicate no thrombin or fibrin formation detectable in all volunteers.