DEPENDENCE OF SOME PRESERVATIVES' ABSORBANCE WITH CALIBRATION CURVE METHOD AND ADDITIVES STANDARDS METHODS BY SPECTROPHOTOMETER

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Abstract

In this proceeding is studied dependence of absorbencies of thimerosal in final product of DTP Vaccine by spectrophotometer [1] with two methods:
1. Calibration Curve Method (C.C.M)
2. Additives Standards Methods (A.S.M).[2]
C.C.M was used for determination of thimerosal during the process of production of DTP vaccine for testing of thimerosal content from Control Laboratory of Vaccine of Institute of Public Health in Albania. This method is not preferred from WHO (World Health Organization), because is not standard method, but is practical and simple method for quality control of vaccine during the process of vaccine production.
The results of experiment show that values of slope are better in case when temperature is maximum(+1), light is minimum(-1) and the time is minimum(-1),[3] but values of correlation coefficient in case of curve of standards additives methods are low and also the results of amount of thimerosal is lower than real amount.
In case when temperature is (+1) and light is (+) and time is (-1) the correlation coefficient are the best but curves have different slopes and results of amount of thimerosal are very low.
The conclusion of this study is that we can not used these methods for determination of thimerosal in final products of DTP vaccine because we can not eliminate the systematic errors.[4]

Key words: vaccine, thimerosal, standard, curve, calibration.

Introduction

Diphtheria, tetanus, and pertussis are serious diseases caused by bacteria. Diphtheria and pertussis are spread from person to person. Tetanus enters the body through cuts or wounds.
Diphtheria causes a thick covering in the back of the throat.
It can lead to breathing problems, paralysis, heart failure, and even death.
Tetanus causes painful tightening of the muscles, usually all over the body.
It can lead to "locking "of the jaw so victim cannot open his mouth or swallow. Tetanus leads to death in up to 2 out of 10 cases.
Pertussis causes coughing spells so bad that is hard for infants to eat, drink, or breathe. These spells can last for weeks. It can lead to pneumonia, seizures (jerking and staring spells), brain damage, and death.
DTaP (Diphteria,tetanus ,and pertussis) vaccine can help prevent these diseases. Children should get 5 doses of DtaP vaccine at each of the following ages:

2 months, 4 months, 6 months 15-18 months, 4-6 years.[5]
During the production of DTaP vaccine used thimerosal to prevent of development bacteria and moulds. Thimerosal is organic compound that content Hg as thiosalycylic ethyl-Hg which is very toxic.
Impurity of vaccine can happen and when we used preservative as thimerosal.
It is condition that amount of thimerosal does not be toxic and does not denature specific substance, because in this way can cut down potency of vaccine during the storage time in recommended temperature.[6]
The amount of thimerosal is no more 0.01%.
If we cut down amount of thimerosal we increase safety but decrease the time of storage of vaccine.[7]

Purpose: Using of standards additives methos is elemination of systematic errors that indicated during the determination of thimerosal with this method in DTP final product.

Instruments and regents.
The measurements of experiments performed with spectrophotometer UNICAM HELIOS, by one radiation, with λ=480 nm.
Reagents:Thiomersal (Merc), Ammonium acetate (CH3COONH4), Dithizone (Perking`s), Nitrique Acide Analypur (LABOSI), Chloroform Analypur.

Materials and methods.
We prepared samples of vaccines according above method [1] .We added thimerosal standard additions with concentration as in down tab.1.

Tab.1

amount of vaccine sample (ml)	1	1	1	1	1
Standards additives thimerosal (mg/l)	0	0.625	1.25	1.875	2.5

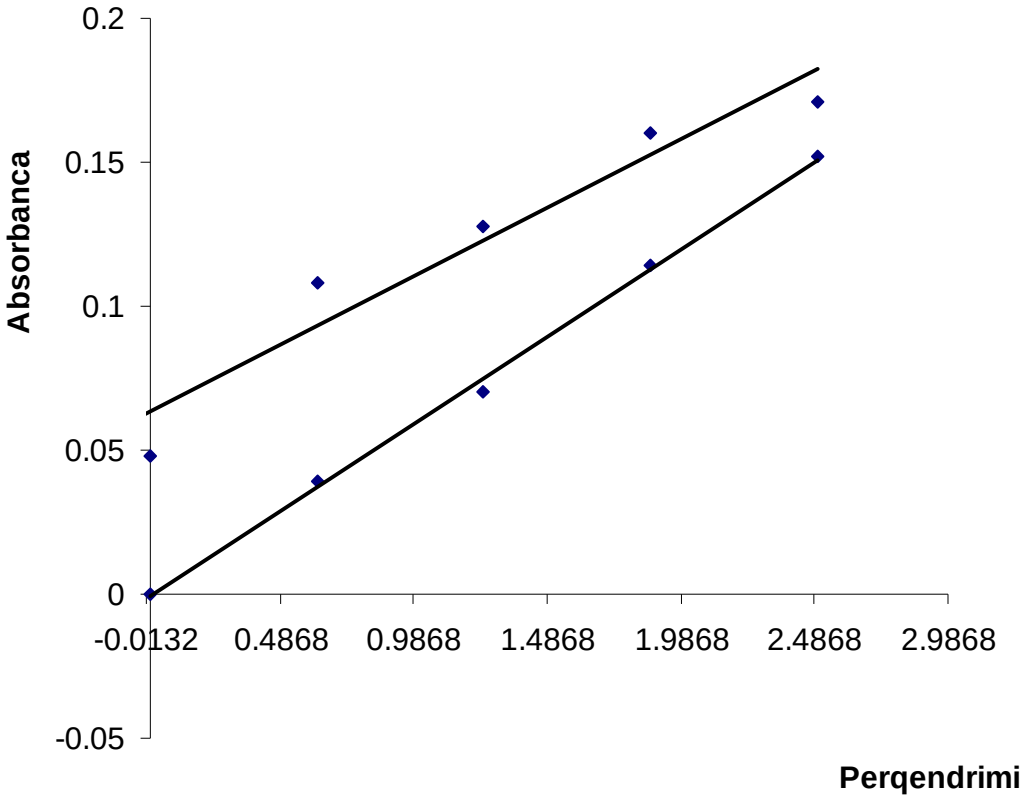
The design of experiments made with Methods of Factorial Design n^m where:
n…number of level.We received 2 levels maximal(+) and minimal level(-).
m…number of factors.We studed 3 factors:
X_1…..temperature
X_2…..light
X_3…..time
We had 8 combinations of factors 2^3.[8]
The results of experiments are in tab.2

Tab.2

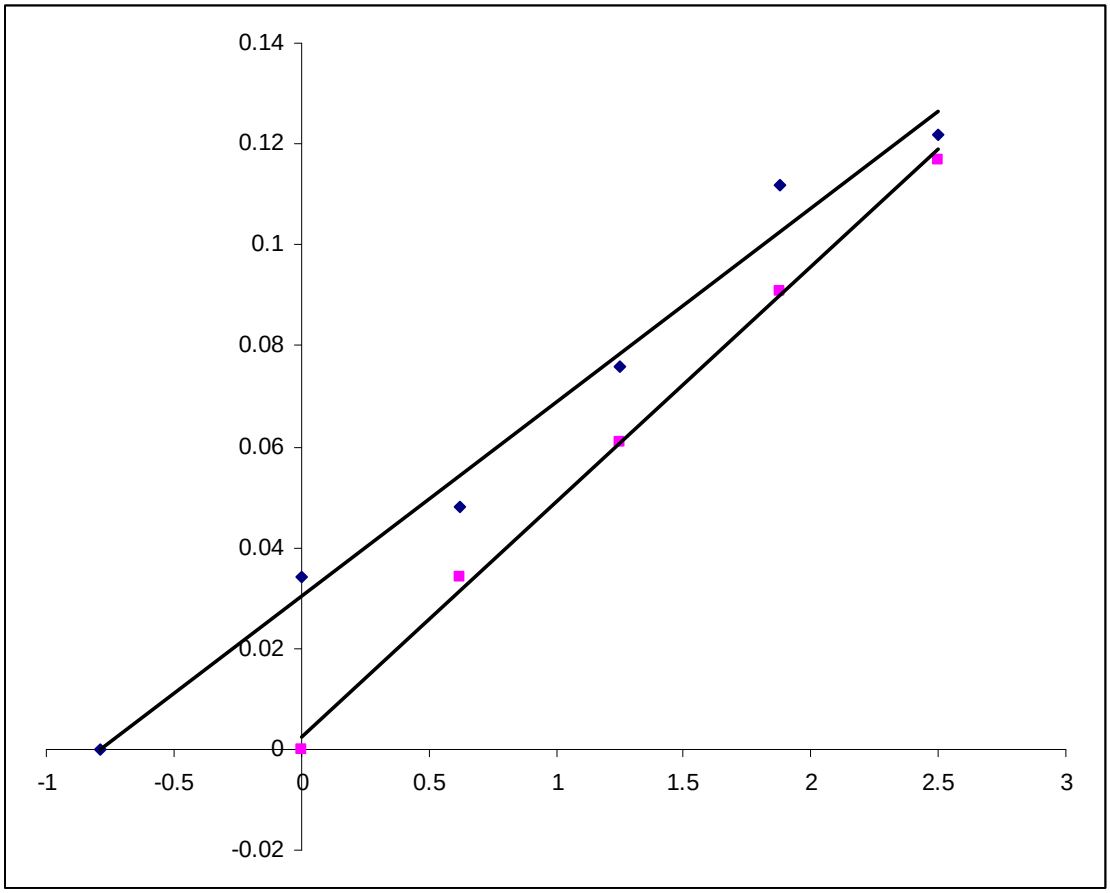
Conditions of experiments			R_1^2	R_2^2	S_1	S_2
X1	**X2**	**X3**				
-1	1	-1	0.9978	09699	0.0606	0.0477
-1	-1	-1	0.998	09845	0.0466	0.0384

-1	1	1	0.9952	0.7963	0.0389	0.0118
1	1	1	0.9807	09557	0.0315	0.0179
1	-1	-1	0.998	0.9712	0.0466	0.036
1	-1	1	0.9904	09162	0.0312	0.0314
1	1	-1	0.9808	09637	0.0347	0.0328
-1	-1	1	0.9934	08866	0.0464	0.0197

Where: R_1^2 is correlation coefficient of calibration curve method.
R_2^2 is correlation coefficient of standards additives method.
S_1 slope of curves of calibration curve methods.
S_2 slope of curves of standards additivies methods.



Graf.1(y absorbance,x concentration)


0.14
0.12
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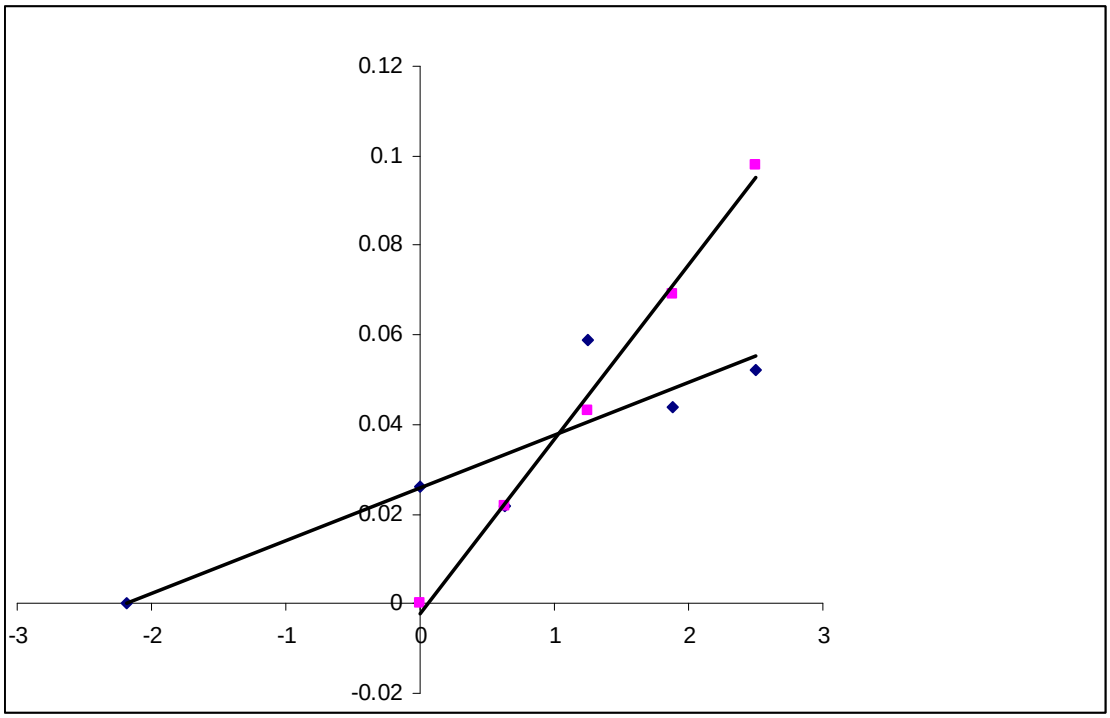
0.5
1
1.5
2
2.5
3

Graf.2


0.12
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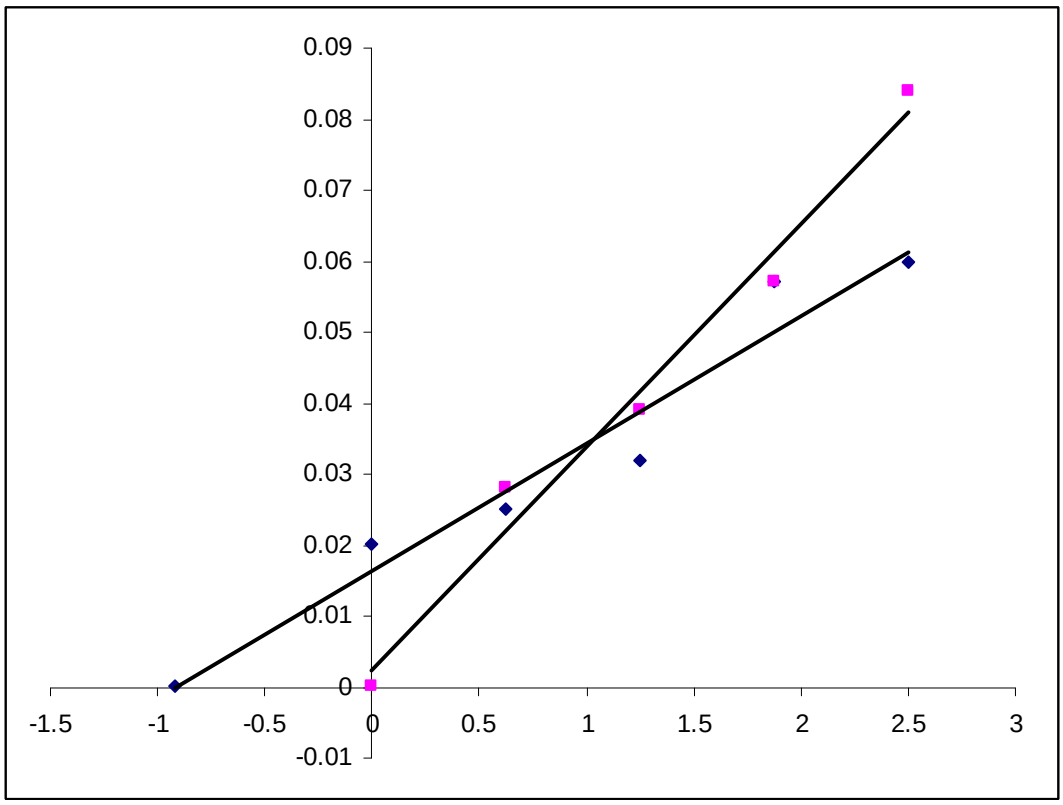
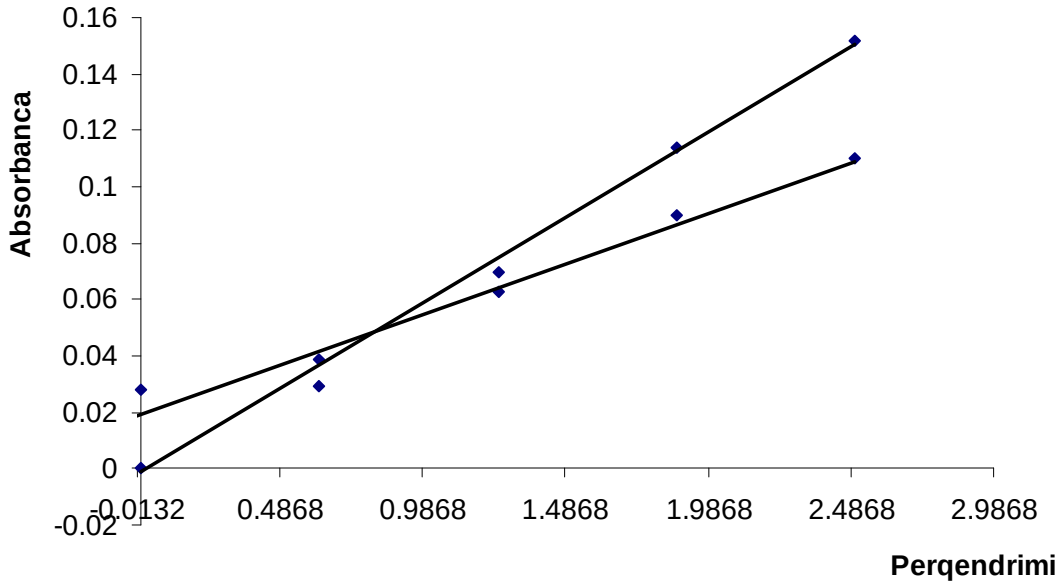
1
2
3

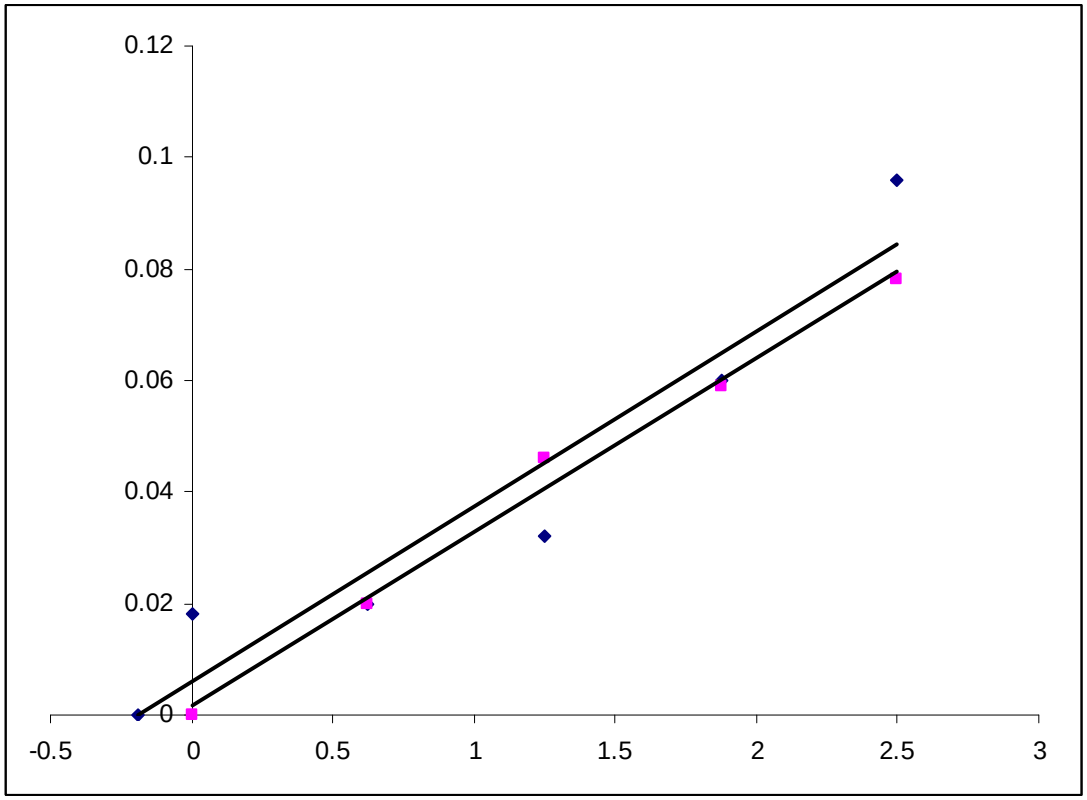
Graf.3



Graf.4



Graf.5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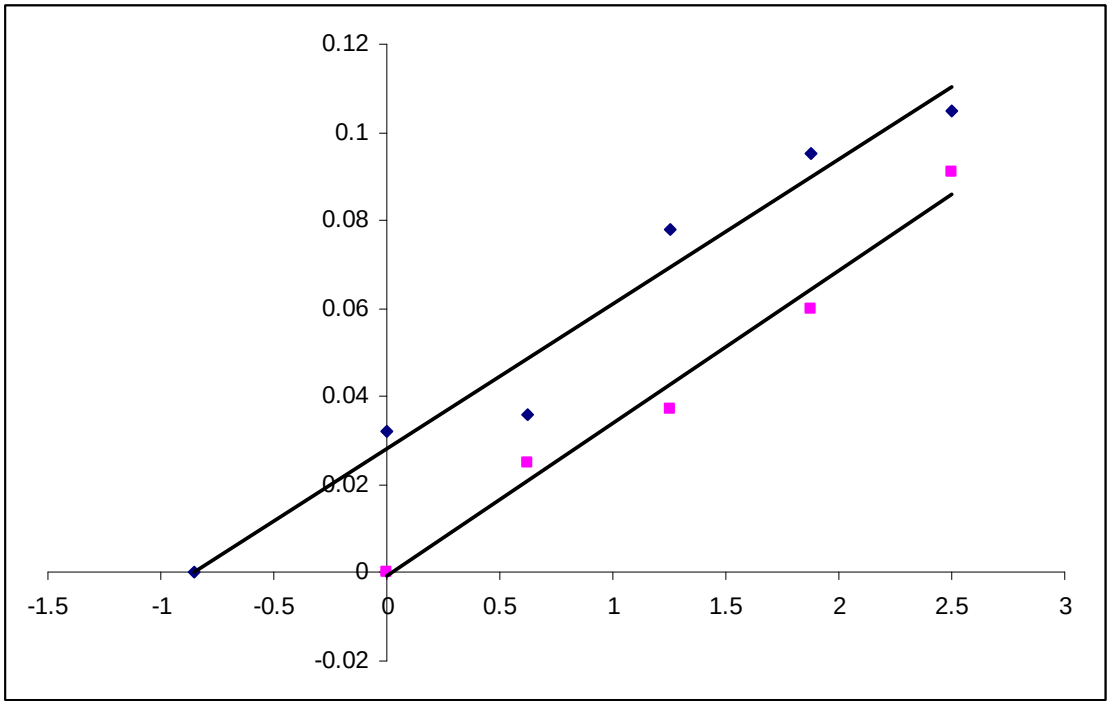
Graf.6


0.12
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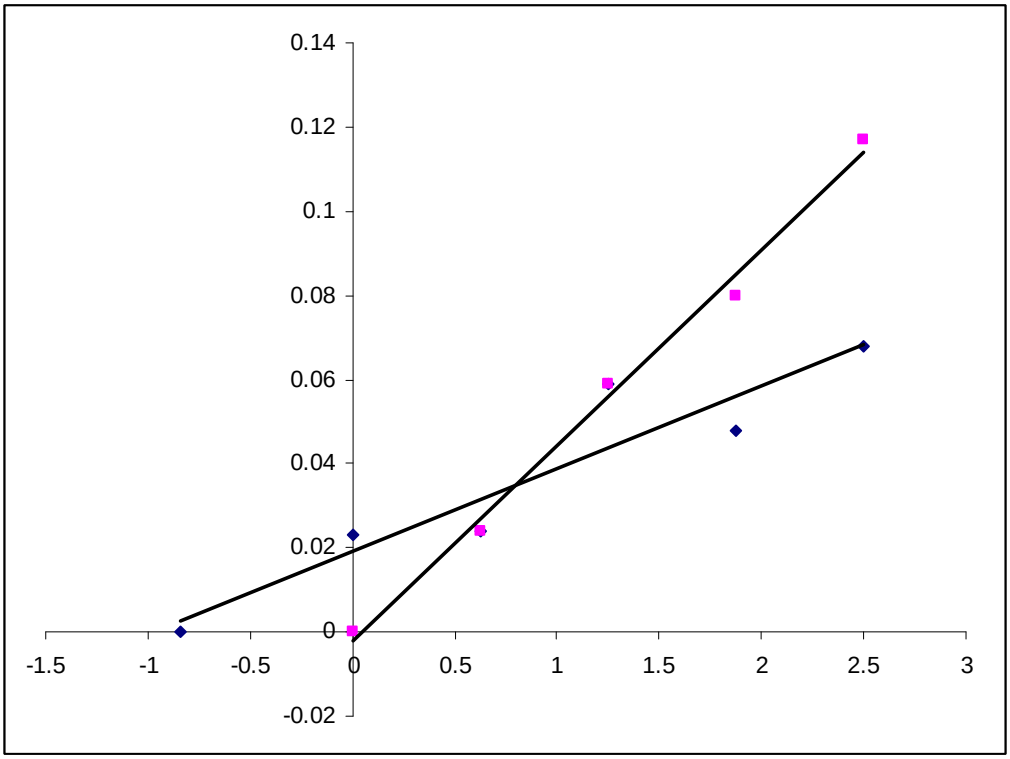
3



Graf.7
Graf.8

Discussion.

The results on Tab.2 show that correlation coefficient has the best value in case when temperature is (+1), light (1), time (-1) but slope of calibration curve method is different from slope of standard additives method and also the amount of thimerosal determinate in vaccine is lower than real amount. The best value of slope are in case when temperature is (+1),light (-1) and time (+1) but the amount of thimerosal determinate is lower than real value.
The standards additives method applied for method of determination of thimerosal with spectrophotometer in this case is not accuracy.
The method of standards additives use when fill up 2 conditions:
1.Concentration of solution (with and without additive)must be in linear zone.
2.Slope(S) must be 0 when C=0 that means calibration curve must pass from origin of axle.[9]

Conclusion.
The method of standards additives is not proper method for determination of thimerosal in final product of DTP vaccine, because this method can not eliminate errors.

Recommendation
We recommended that determination of thimerosal can perform with these methods:
1.Spectrophotometric standard method.
2. HPLC (High Performance Liquid Chromatography).
3.GC (Gas Chromatography),GC-MS(Gas Chromatography-Mass Spectrometry).[10]

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SPEKTROFOTOMETRİK ÜSULLA BƏZİ KONSERVANTLARIN UDULMA DƏRƏCƏSİNİN TƏDQİQİ

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Məqalə diftirit, tetanus və göygskürək (DTP) əleyhinə vaksinlərə qatılan konservantların əyrilərin kolibrə edilməsi və standartların əlavə olunması yolu ilə spektrofotometrik üsulla tədqiqinə həsr olunmuşdur.

Albaniyanın sağlamlıq İnstitutun Yoxlama Laboratoriyasında vaksinlərin hazırlanması prosesində onlara qatılan konservantların keyfiyyətinin bu ysulla yoxlanılmasının həm asan, həm də praktik olmasına baxtayaraq Beynəlxalq Səhiyy Təşkilatı bu üsulları standart kimi qəbul etmədiyindən onlara üstünlük vermir.

Məqalədə alınan nəticələr göstərir ki DTP vaksinlərinə qatılan konservantların keyfiyyət və dərəcəsini bu üsullarla təyinin sistematik xətaları böyük olduğundan həqiqətən bu metodlardan istifadə etmək mümkünsüzdür.

ИССЛЕДОВАНИЕ СТЕПЕНИ ПОГЛОЩЕНИЯ НЕКОТОРЫХ КОНСЕРВАНТОВ С ПОМОЩЬЮ СПЕКТРОФОТОМЕТРА

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В статье исследована степень абсорбции консервантов (Thimerosal) конечным продуктом вакцины от дифтерии, Титануса (столбняка) и коклюша (DTP) с помощью спектрофотометра двумя методами; колибровки кривой и добавления стандартов.

Методы калибровки кривых и добавления стандартов, применяемые контрольной лаборатории вакцин Института здравоохранения Албании, являются простым и практичным методом для контроля качества вакцин во время их производства. Однако, они не принимаются предпочтительными Международной Организацией Здравоохранения, так как не являются стандартными.

В настоящей статье показано, что действительно эти методы не могут быть использованы для определения консервантов в конечной продукции вакцин от DTP, из-за невозможности исключения систематических ошибок.