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# Decrease of Phenolic Preservatives in Insulin Preparations at Different Storing Conditions and after Ending their Expiry Date

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**ABSTRACT**

Determination of phenolic compounds in insulin preparations includes extraction with ethyl acetate (phases ratio organic to aqueous is 1:5 with one batch or 1:10 with two batches) in acidic medium (PH =1.5-2), and then quantification by GC or UV spectrophotometric techniques. This study leads to ensure that the preservatives concentrations are effected by different conditions in the store of insulin preparations. The concentration of methyl paraben was (0.808)mg\ml after expiry date by (150-170) days . When the insulin preparations stored at freezing point for (9-10) days, the concentration of methyl paraben reduced to (0.37)mg\ml, and to (0)mg\ml , after (16-18)days at freezing point . Uv-Vis method was proposed and applied for drawing calibration curve at 272 nm. for m-cresol which is used as preservative in insulin preparations. While methyl paraben was determined by GC technique.

## تقديرانخفاض تركيز المواد الفينولية الحافظة في مستحضرات الأنسولين بعد أنتهاء صلاحيتها بأختلاف ظروف الخزن.

**الخلاصة**

تم أستخلاص المركبات الفينولية الحافظة المستخدمة في مستحضرات الانسولين باستخدام خلات الاثيل كمستخلص (بنسبة طور عضوي الى مائي 1 :5 بدفعة استخلاص واحدة و10:1 بدفعتي استخلاص) وفي وسط حامضي(الاس الهيدروجيني1,5 - 2)ومن ثم تحديدها كميا باستخدام تقنيات كروماتوغرافيا الغازاو مطياف الاشعة المرئية- فوق البنفسجية.

أثبتت هذه الدراسة ان تركيز المواد الحافظة تتاثر بالظروف المختلفة لخزن مستحضرات الانسولين ،حيث انخفض تركيز المثيل بارابين من 1ملغم\ملليترالى 0.808ملغم\ملليتربعدانتهاء مدة صلاحيته بفترة (150-170) يوما . وعندما تم خزن مستحضر الانسولين بدرجة الانجماد لمدة (9-10) ايام انخفض تركيز المثيل بارابين (المادة الحافظة في المستحضر) الى (0.37) ملغم\ملليتر والى (0) ملغم\ملليتربعد فترة (16-18) يوما من الخزن بنفس الظروف.

استخدم مطياف الاشعة المرئية- فوق البنفسجية لرسم منحنى المعايرة لمركب ميتا-كريسول عند طول موجي 272 نانوميترفي حين استخدمت تقنية كروماتوغرافيا الغازللتعيين الكمي

لمركب مثيل بارابين وكل من ال ميتا-كريسول ومثيل بارابين المضافة الـــى مستحضـــرات الانسولين كمواد حافظة.

## INTRODUCTION

Phenolic compounds and their derivatives are widely employed in the pharmaceuticals as preservatives[1]. Sodium benzoate and alkyl esters of p-hydroxybenzoic acid (parabens) including methylparaben and propylparaben are usually used as preservatives to prevent foods, cosmetics, and pharmaceuticals from microbial and fungal attacks [2,3,4] . The antimicrobial activity of parabens increases with increasing length of alkyl chain of ester group but, in practice, shorter esters are commonly used because of their high solubility in water [5]. Methylparaben is a stable, non-volatile compound used as antimicrobial preservative for over 50 years. Methylparaben is readily and completely absorbent through the skin and from the gastrointestinal tract. It is hydrolyzed to p-hydroxybenzoic acid, conjugated, and the conjugates are rapidly excreted in the urine. There is no evidence of accumulation [6]. Determination of these compounds in different preparations requires two steps; extraction and quantification [7,8].

Solvent extraction gas chromatographic method was developed for the determination of m-cresol as preservative in insulin prepns.[9]. The esters of p-hydroxybenzoic acid can be isolated by ether/petroleum ether and determined spectrophotometrically at 255 nm[10]. Methyl paraben which used as preservative in insulin prepns.was determined by solvent extraction gas chromatography [11].

Thankur and Ghosal have reported the preservative in pharmaceuticalformulation[12].Allwood [13] reported the effectivness of preservatives in insulin injections.

## EXPERIMENTAL WORK

A Varian GC-Vista 6000 gas chromatography equipped with both a flame ionization detector and a flame photometric detector. The FID was used during this work. Nitrogen of 99.99% purity was used as carrier gas with an optimum volume flow rate of 30 ml/min. The optimum volume flow-rates of other gases were hydrogen 30 ml/min., and air 300ml/min.

The detector and column inlet temperatures were set at 20°C above the column temp. and isothermal column temperatures 220°C was the most suitable for the analysis.

A linear 1200 paper recorder with capacity 1cm/h was used. Column were made of stainless steel tubing (2*1/8 inch) were packed with 10% OV-101 (100% dimethyl polysiloxanes) liquid stationary phase coated on chromosorb WHP (80-100 mesh). Shimadzu, UV-260, double beam recording UV-VIS spectrophotometer was used for spectrophotometric determinations.

### Materials

All compounds were of high purity purchased from Fluka and BDH and no further purification was needed.

Insulin with m-cresol or methyl paraben was commercially available in disposable vial (volume 10 ml) from NOVO Nordisk, Denmark and Lilly Company France.

**Synthetic Insulin Sample:[14]** it consists of:

**a-**Methylparaben(1mg/ml).
**b-**Sodium acetate(1.7mg/ml).
**c-**Zinc chloride(2.5mg/ml).
**d-**Dibasic sodium metaphosphate(2.5mg/ml).

**Preparation of samples**

Samples of different types of insulin injections were extracted for m-cresol and methyl paraben using ethyl acetate.

The aqueous sample (3-5 ml of insulin injection and 2-5 ml of distilled water) was adjusted to PH=1.5-2 with hydrochloric acid to form a layer down the ethyl acetate. The mixture was shaken vigorously for 2-3 min. at room temp.then set aside for 5 min. and layer was separated and transferred into a 5 ml /vial.

**Analysis**

A standard calibration graph for methyl paraben with ethanol as solvent in Concentration (0.006-0.054)mg/aliquot was set up for analysis methyl paraben by GC technique. A standard calibration graph for m-cresol at 272 nm. was plot.

## RESULTS AND DISCUSSION

In most pharmaceutical preparations, preservationis essential because the excipients, and sometimes the drug itself, may be destroyed by different micro-organisms and consequently the formulation breaks down[15].

Sodium benzoate, substituted phenols and parabens are used as preservative compounds [16].Compounds under studying which used as preservatives were in insulin prepns. are m-cresol (3 mg/ml and methylparaben (1mg/ml).Determination of these compounds consists of two steps:

**Extraction:**

Ethyl acetate was chosen as extractant because of high polarity and easy to separate from an aqueous solution.Both of m-cresol and methylparaben are polar compounds and highly soluble in polar extractant(ethyl acetate).

**Quantification:**

As in fig.(2),analysis of methylparaben by gas chromatography technique requires 10% OV-101 column as stationary phase. Because of low polarity of this column, this choice leads to less interaction with stationary phase and very sharp peak that means best analysis.

All conditions of determination were studied by researcher in former work [11].

Preservatives should be added to formulations,eseptically packed in signal dose vials if the active ingredient(s)does not have bactericidal or bacteriostatic properties or is growth promoting or only is stable in determinate environmental .

Many factors can reduce the effectiveness of preservatives including:

**Ending expiry date of drug:**

After ending the expiry date of insulin prepns. the concn. of methylparaben reduce to(0.808) mg/ml from 1 mg/ml after expiry date by (150-170)days as shown in fig.(6).

This decrement is due to chemical degradation of preservatives and that leads to loss activity of preservation or may be to change optimum medium for drug stability or activity of the antimicrobial preservative [17].

**Freezing at -5°C**

The concn. of methylparaben was reduced to(0.37)mg/ml after storing insulin prepn. at freezing point for (9-10) days ,and to (0)mg/ml after (16-18)days as shown in fig.(7) . To make ensure of these results, the steps of work had been reported with insulin prepn. (Actrapid) which consists of m-cresol as preservative and concn. of m- cresol determined by spectrophotometeric technique.

0.14 ml of organic layer was taken and diluted to 3 ml.The results of absorbance are as shown in table (8).

Many possible explanations may be confident in this case .One of them is the possible formation of a complex containing drug and paraben, in former study the $\lambda_{max}$ of active material is shifted 5nm after freezing calcium leucovorine drug because of interfering with methyl and propyl parabens components [15].

Refrigeration, whilst usually desirable to increase the viscosity of suspension formulation components, or cause the precipitation of active drug or preservative [18].The low aqueous solubility of paraben makes dissolving process very complicated which including addition of alcohol or small volume of heated water and dilution to prevent precipitation of paraben before it cools. Therefore refrigeration may leads toreprecipitate preservative[17].

Casagrande declared that greater activity loss of some drug was detected after freezing for 126 days at 4C°, because of possible interferences of formulations components [19].

## CONCLUSIONS

Methylparaben and m-Cresol are used as preservatives in insulin preparations.Concentrations of these preservatives decrease after ending expiry date of insulin preparations, and after freezing at -4 Cº and less for many days.Therefore it is necessary to store Insulin preparations in suitable conditions. Gas chromatography and Uv-Vis techniques were used as simple and economic technique to determine phenolic preservative materials in Insulin preparations.

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**Table (1) Analysis of insulin preparation (Monotard) using 5% OV-101 column**

| Sample no. | Sample vol., µl | Area under peak, $cm^2$ | methyl paraben Conc.mg/ml | Constant Error(C.E) | Relative Error(R.E) |
|---|---|---|---|---|---|
| 1 | 8 | 0.410 | 1.02 | 0.02 | 1.96 |
| 2 | 8 | 0.406 | 1.007 | 0.007 | 0.695 |
| 3 | 8 | 0.40 | 0.987 | -0.013 | 1.317 |
| 4 | 6 | 0.33 | 1.004 | 0.004 | 0.398 |
| 5 | 5 | 0.294 | 1.012 | 0.012 | 1.185 |
| 6 | 5 | 0.29 | 0.991 | -0.009 | 0.908 |

**Table(2) Analysis of synthetic sample using 5% OV-101 column.**

| Sample no. | Sample vol., µl | Area under peak, $cm^2$ | methyl paraben Conc.mg/ml | C.E | R.E |
|---|---|---|---|---|---|
| 1 | 8 | 0.405 | 1.004 | 0.004 | 0.398 |
| 2 | 8 | 0.407 | 1.01 | 0.01 | 0.99 |
| 3 | 6 | 0.33 | 1.004 | 0.004 | 0.398 |
| 4 | 6 | 0.329 | 0.999 | -0.001 | 0.10 |
| 5 | 5 | 0.291 | 0.996 | -0.04 | 0.40 |
| | | | Av.= 1.003 | | |

**Table (3) Analysis of insulin preparation (Humulin) using 10% OV-101 column with 2.636 Relative Standard Deviation (R.S.D %).**

| Sample no. | Sample vol., µl | Area under peak, $cm^2$ | methyl paraben Conc.mg/ml | C.E | R.E |
|---|---|---|---|---|---|
| 1 | 10 | 0.590 | 0.9933 | -0.0066 | 0.664 |
| 2 | 10 | 0.600 | 1.020 | 0.020 | 1.960 |
| 3 | 7 | 0.480 | 0.9996 | -0.0004 | 0.040 |
| 4 | 7 | 0.488 | 1.030 | 0.030 | 2.912 |
| 5 | 5 | 0.398 | 0.962 | 0.038 | 3.950 |
| | | | Av.=1.0009 | | |

**Table (4 )Analysis of synthetic sample using 10% OV-101 column with 1.212 R.S.D.%.**

| Sample no. | Sample vol., µl | Area under peak, $cm^2$ | methyl paraben Conc.mg/ml | C.E | R.E |
|---|---|---|---|---|---|
| 1 | 5 | 0.4035 | 0.991 | -0.009 | 0.8966 |
| 2 | 5 | 0.408 | 1.015 | 0.015 | 1.494 |
| 3 | 7 | 0.483 | 1.011 | 0.011 | 1.096 |
| 4 | 7 | 0.480 | 0.9996 | -0.0004 | 0.0396 |
| 5 | 9 | 0.552 | 0.991 | -0.0097 | 0.966 |
| 6 | 9 | 0.560 | 1.0148 | 0.0148 | 1.474 |
| | | | Av.=1.0037 | | |

**Table (5)Analysis of insulin prepn.(Monotard) after ending its expiry date by (40-60) days**

| Sample no. | Sample vol. | Area under peak,$cm^2$ | Concn. of methyl paraben,mg/ml | R.S.D |
|---|---|---|---|---|
| 1 | 5 | 0.329 | 0.929 | 0.324 |
| 2 | 7 | 0.460 | 0.923 | |
| 3 | 9 | 0.530 | 0.926 | |

**Table (6)Analysis of insulin prepn. (Monotard) after ending its expiry date by (150-170) days**

| Sample no. | Sample vol. | Area under peak,$cm^2$ | Concn. of methyl paraben,mg/ml | R.S.D |
|---|---|---|---|---|
| 1 | 5 | 0.368 | 0.801 | 0.961 |
| 2 | 7 | 0.432 | 0.8164 | |
| 3 | 9 | 0.490 | 0.807 | |

**Table (7)Analysis of insulin prepn. (Monotard) after storing at freezing point for (6-7) days**

| Sample no. | Sample vol. | Area under peak,$cm^2$ | Concn. of methyl paraben,mg/ml | R.S.D |
|---|---|---|---|---|
| 1 | 9 | 0.38 | 0.481 | 1.819 |
| 2 | 7 | 0.34 | 0.466 | |
| 3 | 5 | 0.308 | 0.481 | |

**Table (8)Analysis of insulin prepn. (Monotard) after storing at freezing point for (9-10) days**

| Sample no. | Sample vol. | Area under peak,$cm^2$ | Concn. of methyl paraben,mg/ml | R.S.D |
|---|---|---|---|---|
| 1 | 9 | 0.34 | 0.362 | 1.88 |
| 2 | 7 | 0.316 | 0.374 | |
| 3 | 5 | 0.288 | 0.374 | |

**Table (9) Analysis of insulin prepn. ( at different storing conditions) using spectrophotometric technique.**

| | Concn. of m-cresol (mg/ml) | Absorbance |
|---|---|---|
| **Insulin prepn. before exp. Date** | 2.907 | 1.912 |
| **After ending exp. date by (20-30) days** | 2.632 | 1.734 |
| **After ending exp. date by (150-170) days** | 2.192 | 1.449 |

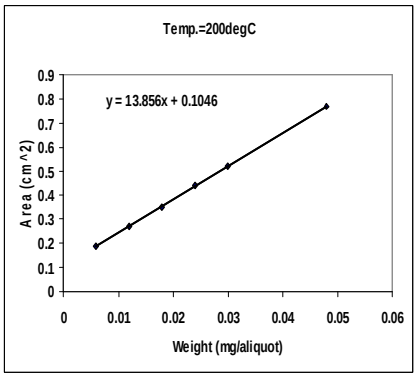


**Figure (1) Standard calibration curve for methylparaben on 5% , 0V-101 at 200 C.**

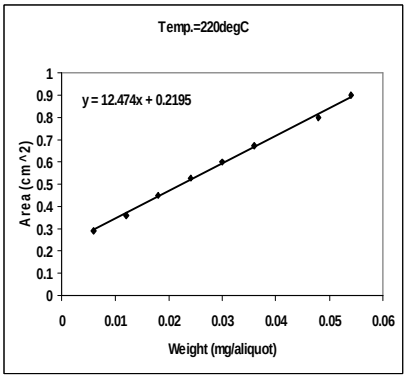


**Figure (2) Standard calibration curve for methylparaben on 10% , 0V-101 at 220 C.**

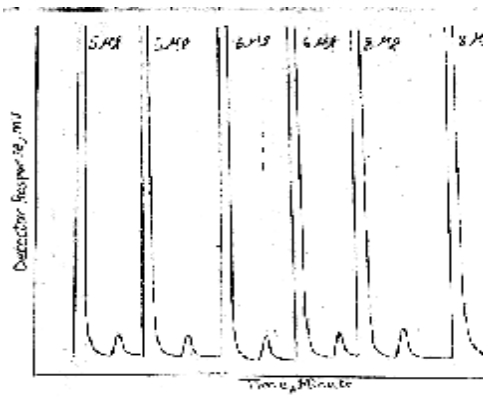

**Figure (3)Chromatograms of methyl paraben extracted in one batch from an insulin injection (monotard) using Ethyl acetate on 5%, OV-101 at 200C°**

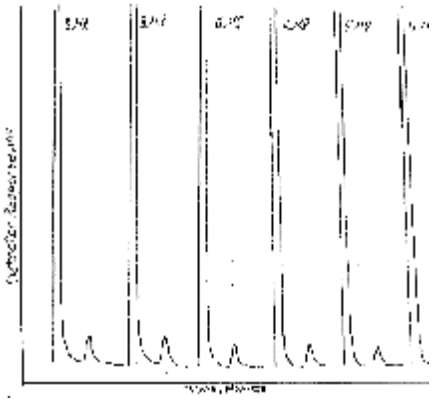

**Figure (4) Chromatograms of methyl paraben extracted in one batch from synthetic sample using Ethyl acetate on 5%, OV-101 at 200C°**

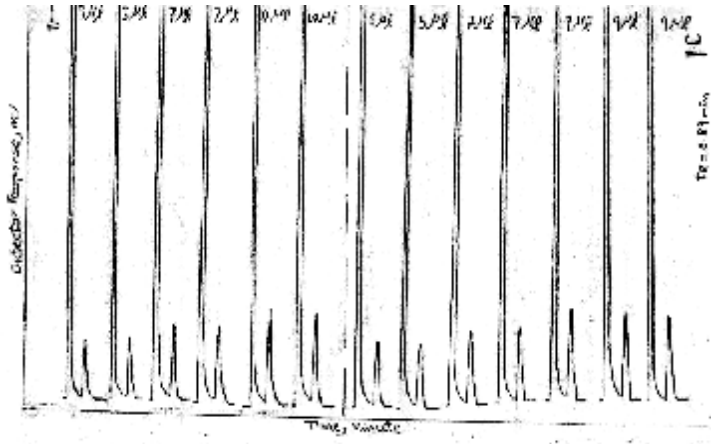

**Figure (5) Chromatograms of methyl paraben extracted in two batches using Ethyl acetate from an insulin injection (Humulin), (a) from synthetic sample, (b) on 10%, OV-101 at 220C°.**

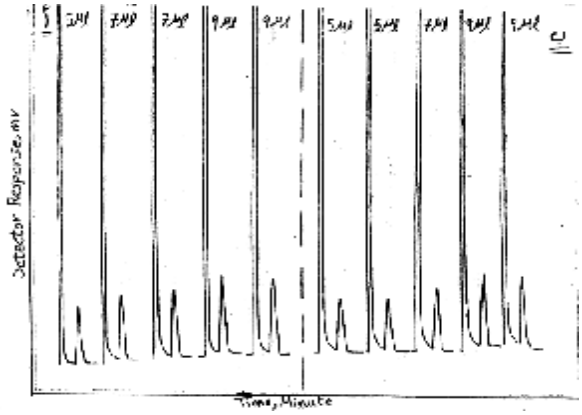


**Figure (6) Chromatograms of methyl paraben extracted in two batches using Ethyl acetate from an insulin injection (Humulin) after its expiry date with (40-60) days in (a), (150-170 )days in (b)on 10% OV-10 1 at 220C$^{o}$ which was stored at freezing point for (6-7) days in (a) and for (9-10) days in (b)on 10%, OV-101 at 220C$^{o}$.**

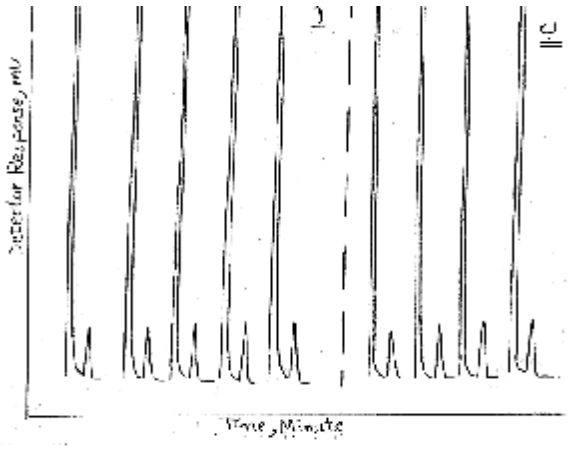


**Figure (7) Chromatograms of methyl paraben extracted in two batches using Ethyl acetate from an insulin injection (Monotard) which was stored at freezing point for (6-7) days in (a) and for (9-10) days in (b)on 10%, OV-101 at 220C$^{o}$.**

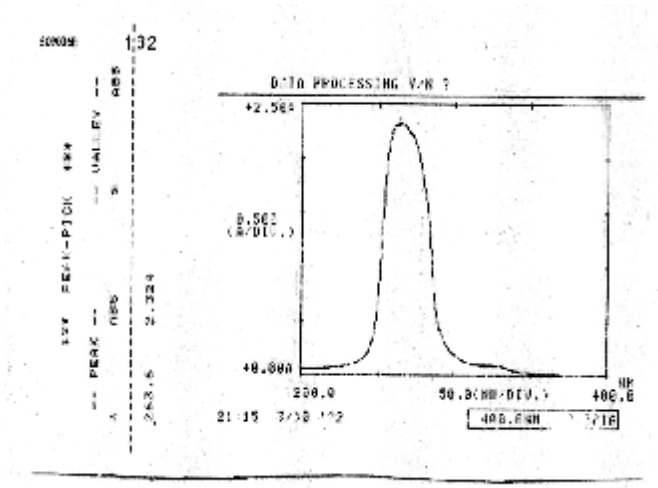

**Figure (8)Ultraviolet absorption for methyl paraben from an insulin injection (Monotard) extracted using ethyl acetate .**

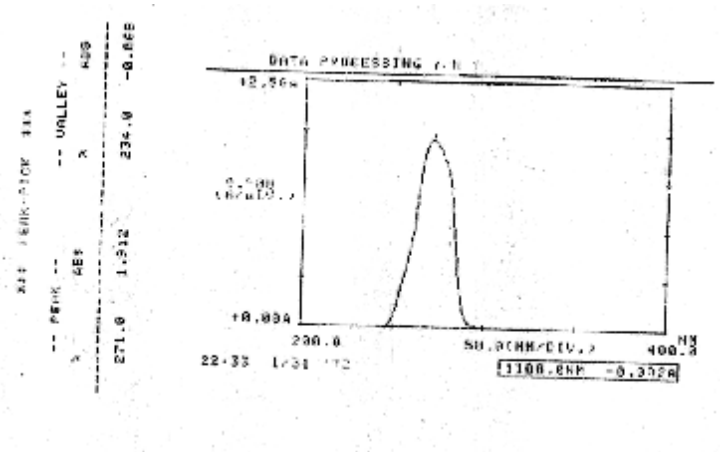

**Figure (9)Ultraviolet absorption for meta-cresol from an insulin injection (Actrapid) extracted using ethyl acetate**

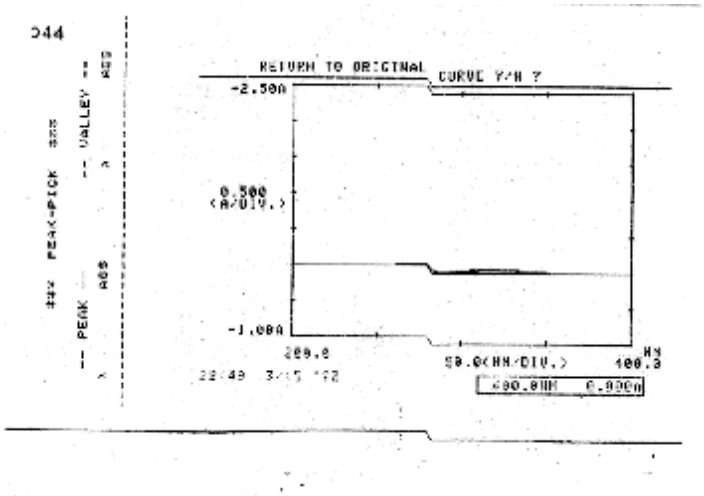


**Figure (10)Ultraviolet absorption for meta-cresol from an insulin injection (Monotard) extracted using ethyl acetate after storing it at freezing point for 16 days.**

**C=K*Abs+b, K=0.0721, b=-0.00022**

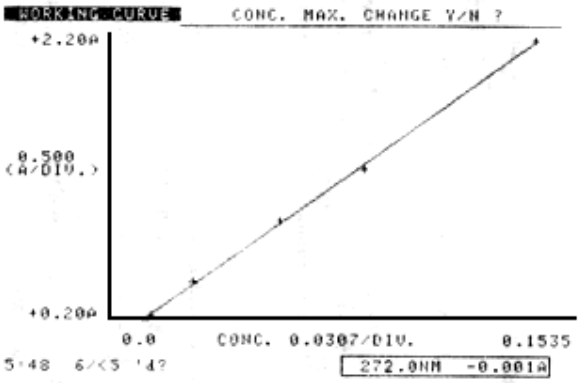


**Figure (11)Standard calibration with curve for m-cresol by ultraviolet technique.**