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Spectrophotometric Determination of Methyl Paraben in Pharmaceutical Formulations by Oxidative Coupling Reaction

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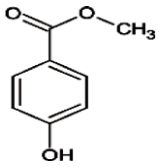
Abstract
A simple and sensitive spectrophotometric method was developed for the determination of Methyl Paraben via oxidative coupling with the 2,4-Dinitrophynel hydrazine in the presence of N-bromosuccinimid in a basic medium, Absorbance of the obtained coloured products was measured at the corresponding optimum wavelengths (600 nm), Beer's law is obeyed over the concentration of 2.0-20 μg.ml^{-1}, and The molar absorptivity of (6253 L.mole^{-1}.cm^{-1}), Sandell's sensitity index of (0.02433 μg.cm^{-2}). The detection limits were (0.34 μg.ml^{-1}), with a linear regression correlation coefficient of (0.9947) and recovery range from (98.96–103%). The proposed method has been applied successfully to determine Methyl Paraben in pharmaceutical preparations and cosmetic formulations.

Keywords: Spectrophotometric, Methyl Paraben

1. Introduction
The scientific name for the Methyl Paraben (Methyl 4-hydroxybenzoate, Methyle p-hydroxy benzoate ,Methyl parahydroxy benzoate) [1] . Its composition formula:



Fig(1): Chemical structure for Methyl Paraben $C_8H_8O_3$, M.wt= 152.15 g/mol [2]

Methyl Paraben, It is a crystalline powder fine white solid which is slightly soluble in water and dissolves well in alcohol and ethanol [3], and Its melts at 68.8°C [4]. Instance paraben is now used as a preservative in food, pharmaceuticals and cosmetics[5],dayily used the productes that continue of methyl paraben may be caused in the future breast cancer[6]. Different analytical method have been used for the determination of MP such as Spectrophotometric method[7], HPLC[8], electrochemical method[9], flow-injection technique[10], and Detection chemical fluorescence[11].

2.Experimental
2-1 Apparatus:
Spectrophotometric measurements have been preform using GBC UV-Visible – Cintra 6.

2-2 Reagent and chemicals used:
All chemicals and analytical reagent used in this reseach are purity and equipped by companies BDF, Merck and SDI.

2-3 Preparation of solution:
A- Standard Methyl Paraben solution, (250 μg.ml^{-1}):
This solution was attended by dissolving (0.025gm), in 2 ml of ethanol and diluted to 100ml with distilled water in a volumetric flask for Methyl solution.

B- 2,4- Dinitropheynel hydrazine reagent solution (0.005M):
This solution was attended by dissolving 0.09907 grams of powder in 1 ml of Sulphuric Acid and then complete the volume to 100 ml with distilled water.

C- N-Bromosuccinimide solution (0.01M):
This solution is prepared by dissolving 0.178 grams of N-Bromosuccinimide in 4 ml of ethanol and then complete the volume to 100 ml with distilled water .

D- NaOH solution (1M) Approxmitelly:
This solution was attended by dissolving 4gm of sodium hydroxide with distilled water and then complete the volume to 100 mL with distill water in a volumetric flask.

E-Solution of MP Syrup formulation (500 μg/ml) :
The solution has been prepared by taken 5 ml of Cyprodien Syrup that containing(0.01-0.04 μg.ml^{-1}) of Methyl Paraben, the volume is completed with distilled water in volumetric flask of 100ml and then taken 50 ml and added to 100 ml volumetric flask completed with distilled water, which gives concentration of (500μg.ml^{-1}) .

3- Result and discussion
A (1.5ml) of N- bromosuccinimide(0.01M) is added to (2ml) of Standard MP solution in the presence of (1.5ml) of 2,4- Dinitropheynel hydrazine (0.005M) solution in basic medium (2.3ml of 1 M, NaOH), diluted with distilled water in (25 ml) volumetric flask, agreen-blue product for MP. Absorption spectrum of the Colored dye against its corresponding to blank reagent maximum absorption λ_{max} at 600 nm in contrast to blank reagent which shows a few absorbance at this wavelength.

4- Optimization of the experimental condition
The effect of various variable on the Color intensity of 2ml of standard Methyl Paraben solution (250 μg /ml), 1.5ml of n-bromosuccinimide and 1.5ml of 2,4-dinitropheynel hydrazine and (2.3ml) of NaOH (1M), was studied to establish the optimum conditions.

4-1 Selected the best of oxidizing agent:

Oxidizing agent 1×10^{-2}M	Absorbance		λ_{max} (nm)
	Blank	Sample	
KSCN	0.058	0.114	330
$K_2S_2O_7$	0.077	0.125	309
$FeCL_3$	0.124	0.108	413
NBS	0.034	0.324	600

4-2 Effect of amount of oxidizing agent:

This study was conducted to select the best amoumt of oxidizing agent n-bromosuccinimide (0.01M) by adding different (0.8-2.0 ml) volumes of oxidizing agent to volumetric flasks containing 2ml of methyl paraben (250 µg /ml) then addition of 1.5ml of reagent 2,4- dinitropheynel hydrazine and (2.3ml) of NaOH (1M) and the volume was completed to (25ml) with distilled water.

Table (1): Effect of the amount of oxidizing agent

Ml of N- bromosccinimied (0.01M)	Abs (nm)
0.8	0.0283
1.0	0.0850
1.2	0.1742
1.3	0.2164
1.4	0.2858
1.5	**0.3517**
2.0	0.0785

the result are shown the volume (1.5 ml) is the optimum amount because of highest absorbance, so it was used in subsequent experiments.

4-3 Effect of the amount of coupling reagent:

effect of the amount of coupling reagent was studied by adding different volumes (0.6-1.7ml) of 2,4-dinitropheynel hydrazine (0.005M) to the volumetric flasks containing 2ml of methyl paraben (250 µg /ml) and (1.5ml) of N-bromosuccinimide (0.01M), then addition of (2.3ml) of 1M NaOH, and the and the volume was completed to (25ml) with distilled water.

Table (2): Effect of the amount of coupling reagent

Ml of 2,4-dinitropheynel hydrazine	Abs (nm)	ml of 2,4- dinitropheynel hydrazine	Abs (nm)
0.6	0.0779	1.0	0.3117
0.7	0.1320	1.2	0.3156
0.8	0.2342	**1.5**	**0.3473**
0.9	0.2517	1.7	0.3030

the result are shown the volume (1.5) ml is the optimum amount because of highest absorbance, so it was used in subsequent experiments.

4-4 Effect of the base:

The effect of base was studied by adding (0.8-3.0 ml) of (1M) NaOH solution . the best pH is found 12.14 , and (2.3ml) of NaOH , was adopted in subsequent experiments. The result are shown in table.

Table (3): Effect of base

pH	ml of NaOH	Abs (nm)	ml of NaOH	pH	Abs (nm)
11.6	0.8	0.1083	2.0	12.06	0.3341
11.77	1.0	0.18911	2.3	12.14	0.3556
11.85	1.3	0.2460	2.5	12.22	0.2965
11.95	1.5	0.3032	3.0	12.24	0.2955

4-5 Effect of Oxidation time:

It has been studied Oxidation time , by taking a series of volumetric flask(25 mL) and added (2 ml) of Methyl paraben (250 µg/ ml) and added (1.5 ml) of oxidant N- Bromosuccinimid (0.01 M) , then (1.5 ml) of the reagent 2-4 dinitropheynel hydrazine (0.005M), then added (2.3 ml) of NaOH (1M) , was dilution with distilled water in volumetric flask (25 mL) limit mark and measure the absorbance of the solutions at the wavelength of (600 nm) versus blank, the result are shown in the Table(4).

Table(4): Effect of Oxidation time.

Tim(min)	5	10	15	20	25	30	35	40	50
Abs(nm)	0.3819	0.3235	0.2693	0.2688	0.2645	0.2485	0.2306	0.2001	0.1898

Table(4) shows that 10 min is sufficient for the oxidation to be completed, so it adopted in subsequent experiments.

4-6 Order of additions:

The effect of different orders of addition on the absorption of colored product have been studied. It is found that the addition sequence (3) achieves a higher absorption of colored product, so it was adopted in subsequent (experiments and the result are show in Table (5))

Table (5) Order of additions

Order number	Order of addition	Abs
1	S+O+B+R	0.0551
2	S+R+B+O	0.0144
3	S+O+R+B	0.3261
4	S+R+O+B	0.0439

4-7 Effect of temperature:

The effect of temperature on the absorption was studied and the temperatures ranging between (5-50 °C) ,on the absorption of the colored product formed, as shown in the table (6).

Table (6) Effect of temperature

Temperature C°	5	10	15	20	**25**	30	35	40	50
Abs(nm)	0.2356	0.2676	0.2666	0.2824	**0.3599**	0.2782	0.2500	0.1768	0.1640

4-8 Effect of time on stability of the colored product:

The stability time of the formed colored product was studied by taking 2ml of methyl paraben(250µg/ml) with addition (1.5ml) of n-bromosuccinimied , then (1.5ml) 2,4-dinitrophenyl hydrazine (0.005M) and (2.3 ml) NaOH solution (1M). the volume is completed to(25 ml) in a volumetric flasks with distilled water, the value of the absorption of the colored product remain for not less than (50) min. The results are show on Table (7).

Table (7): Effect of time on stability of the colored product

Time (min)	5	10	15	20	25	30	40	50	60
Abs(nm).	0.3719	0.2986	0.2971	0.2946	0.2968	0.2967	0.2843	0.2645	0.2306

4-9 Effect of solvent:

The effect of solvent was studied on the formed colored product, the dilution was carried out by different solvent instead of water. The results are shown on Tablet(8).

Table (8): Effect of the solvent

Solvent	**Absorbance**	**λ_{max} nm**	**Solvent**	**Absorbance**	**λ_{max} nm**
Water	**0.370**	**600**	Propanol	0.286	550
Acetone	0.621	530	Ethanol	0.421	560

The result shown in table(8) indicate that the water is a good medium for reaction and give good absorption value at the wavelength of 600 nm, it used as the best solvent in the subsequent experiments.

5- Final absorption spectrum

The spectrum of the formed colored product by coupling of MP with 2,4- dinitrophenyel hydrazine in the presence of N-bromosuccinimied in basic medium pH= 12.14 at 25 C^0 against its corresponding reagent blank show a maxim absorption at 600 nm in contrast to the blank reaction of a few absorbance at λ_{max} .

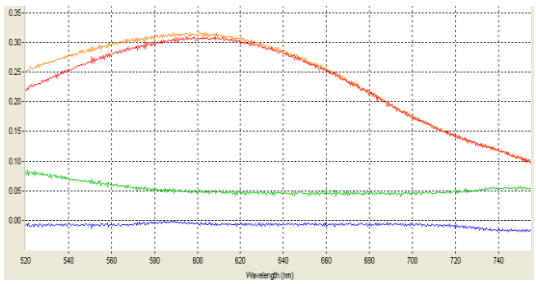


Fig (2): Final absorption spectrum of the determntion Methyl Paraben

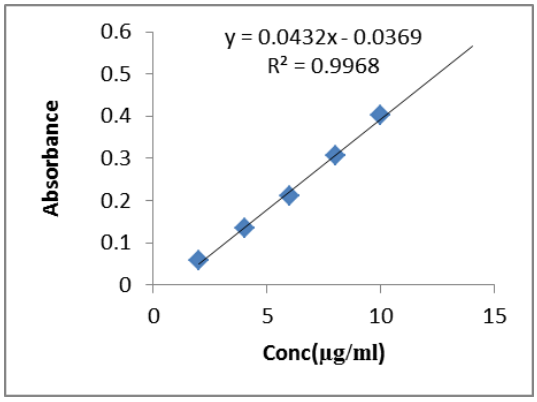
A: Absorption spectrum of Colored Product versus blank reaction.

B: Absorption spectrum of Colored Product versus distilled water.

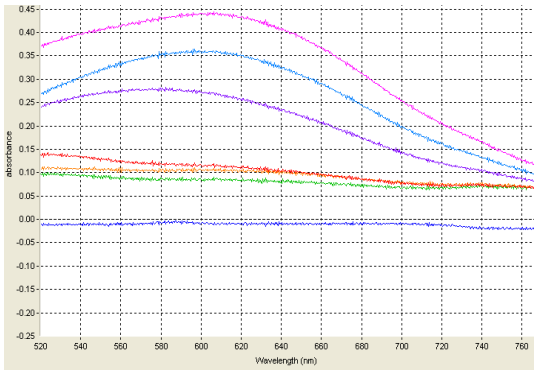
C: Absorption of blank reagent versus distilled water.

6-procedure for construction of calibration curve

Calibration curve was preparation by adding to a series of volumetric flasks (25 ml) , (0.2-2.0 ml) (250 µg /ml) of Methyl Paraben were transferred, (1.5ml) of N-bromosuccinimied (0.01M) and (1.5ml) of 2,4-dinitrophenyl hydrazine (0.005M), after that the solution were left for (5 min) to complete the reaction, then the added (2.3 ml) of NaOH (1M), the volume is completed to (25 ml) in a volumetric flasks with distilled water. The absorbance was measure at 600 nm in against the blank reaction. Fig (2), (3) illustrates that the calibration curve and absorption spectrum of is linear over the concentration range of (2 – 20 µg .ml^{-1}) while higher concentration show a negative deviation form bee's law. The molar absorptivity value is (6.253×10^3 Liter/mol.cm^{-1}) and the Sandell's sensitity index of(0.02433 µg.cm^{-2}) .



Fig(3): Calibration curve for Determination of Methyl Paraben



Fig(4) Absorption spectrum of concertation (2-20 µg.ml^{-1}) for MP

7- Accuracy and precision

Accuracy and precision were studied by measuring absorption the drug (10, 20 µg .ml^{-1}) within the limits of bee's law, the average recovery (99.17 %) and the (>1.42%) indicate that the method is of high

Accuracy and precision. The results are shown in Table (9).

Table (9): Accuracy and precision

Conc. of MP µg/ml	RE %	Recovery %	average recovery%	RSD %
8	1.25–	98.75	99.17	1.42
10	0.4 –	99.6		0.95

8- Detection limit

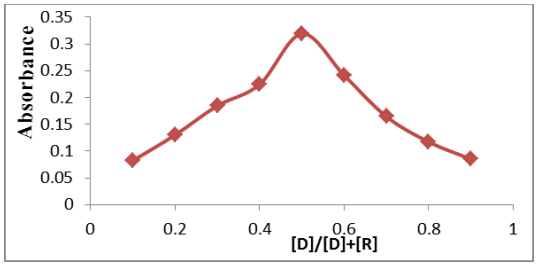
Detection limit was calculate by measuring the absorption for the lower concentration (2 µg .ml^{-1}) at optimal condition at 600 nm. The result are show in Table (10).

Table (10): Detection limit

Conc. Of MP µg /ml	$\bar{X}$	S	D.L µg/ml
2	0.0882	0.005	0.34

9- The nature of the formed product

To now The nature of the formed (green-blue) color product,Job's method and molar method were applied.in both methods the concentration of each of the standard Methyl Paraben solution and 2,4-dinitrophenyl hydrazine reagent solution. In Job's method, in a series of volumetric flasks (25 ml), different volume of the drug solution ranging from (0.1- 0.9 ml) and defferent volumes (0.9 -0.1 ml) of reagent solution were mixed. A (1.5 ml) of N-bromosuccinimied and (2.3 ml) of NaOH solution were added and the volumes were completed to the mark with distilled water. The absorbance was measured at 600 nm in against the blank reaction. Fig(4) show that the ratio is 1:1.



Fig(5): Job's method of formed product by oxidative coupling of MP with 2,4- DNPH

In molar ratio method, 2 ml of standard drug solution added in a series of volumetric flasks (25 ml) were teransferred and different volumes (0.2 – 2.0 ml) of 2,4- dinitrophenyl hydrazine reagent solution, (1.5 ml) of N-bromosuccinimied and (2.3 ml) of NaOH (1M) solution were added. the volumes were completed to the mark with distilled water. The absorbance was measured at 600 nm in against the blank reagent. Molar ratio was found to be 1:1 the results are shown in Fig (5) which is in agreement the Job's method result.

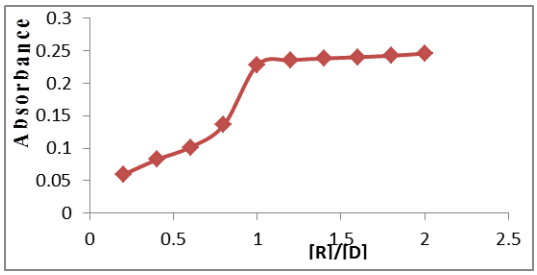
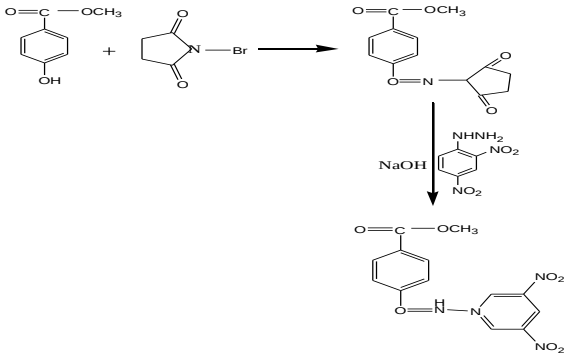


Fig (6): Molar ratio method of formed product by oxidative coupling of MP with 2,4- DNPH reagent .

The proposed equation for for reaction be written of follows:



10- Application

10-1 Direct method:

In this method, different volumes (1.0 – 2.0ml) of a pharmaceutical formulation (500 µg /ml) were transferred to (25 ml) volumetric flasks and the resulting concentration (10 , 15) and were treated as in construction of calibration curve. The absorbance was measured are shown at 600 nm for five times. Recovery and RSD were calculated and the results are shown in Table (11).

Table (11): Direct method

Conc. of MP µg/ml	RE %	Recovery %	Average recovery %	RSD %
10	0.6	99.92	99.44	0.66
15	0.4	98.96		1.42

Results from the above table the success of the proposed method to estimate Methyl Paraben in in the pharmaceutical preparation containing it, the value of recovery of 99.44% in the product Syprodin syrup that unknown concentration.

10-2 Standard addition method:

For the determination of Methyl Paraben in the pharmaceutical preparation and to prove that the way free from interference applied the standerd method to estimate the added Methyl Paraben in pharmaceutical preparation Cyprodien Syrup. It included the way Add fixed amount (1.0 , 1.5 ml) concentration of (500 µg .ml^{-1}) to a series of volumetric flasks (25ml) , was added volumes increased (0.5, 1.0, 1.5, 2.0, 2.5) of the solution Methyl Paraben record a concentration of (500 µg .ml^{-1}) , was the treatment of above solution work the same method used when preparing the calibration curve, has been measuring the absorption of all solution compared to the solution when the

picture wavelength 600 nm, and the results are shown in Table (12) and Fig (6).

Table (12): Standard additions method

Type of Drug	MP µg /ml present	MP µg /ml measured	Recovery (%)
Cyprodin Syrup	10	0.8	102
	15	2.4	103

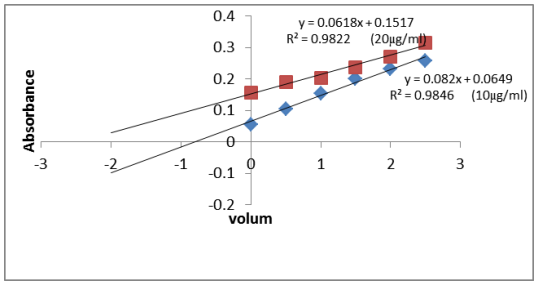


Fig (7): Standard additions method

Results can be seen from the table (12) that the method of standard addition and agree well with the direct method whithin the acceptable range of error, indicating that the way satisfactory and free from interference.

11- Conclusions

The result obtained confirm that the proposed method is simple, rapid and a good sensitivity for the Determination of Methyl Paraben . The method is based on oxidative coupling between MP and 2,4-dinitrophenyln hydrazine reagent in presence of N-bromosuccinimied in basic medium to form (green-blue) colored dye which in water soluble, stable and show a maximum absorption at 600nm. This method does not require temperature control, used organic solvent (methanol), it can be applied successfully for determntion of methyl paraben in pharmaceutical formulation with recovery not less than 98.96%.

Reference

1- The American extra pharmacopoeia; 2005 David Winston , Herbalist AHG.

2- F. Giordano and R. Bettini, C. Donini, A. Gazzaniga, M. R. Caira, G.C. Zhang and D. J. Grant, "Physical properties of parabens and their mixtures: solubility in water, thermal behavior, and crystal structures", J. Pharm. Sci., 88(11), 1210-6 (1999).

3- S. Taylor and G. Burdock, "Evaluation of the health aspects of Methyl Paraben areview of the Published literature", J. food . chem. Toxi. , 40(10), 1335-1373 (2002).

4- M. Anderson and K. Kitagaito, S. Lee and E. Martinez; Paraben , CHEM355 – Organic Chemistry II, 1-13, (2011).

5- R. EJ, Parker and J, Odum,"Some alkyl hydroxy benzoate preservatives(parabens)a are estrogenic", J. Toxicol Appl pharma.,153(1), 9-12 (1988).

6- P. W.,Harvey,"Paraben, Oestrogen under arm cosmetics and breast cancer. Aperspective on hypothesis", J. Appl. Toxi., 23(5), 285-288 (2003).

7- S. Dhahir and H. Hussien, "Spectrophotometric Determination of methyl paraben in pure and pharmaceutical Oral Solution", J. Advan. Nat. Sci., 6(4), (2013).

8- B. Saad and Md. Fazlul Bari, M. Idiris Saleh and K. Ahmed, Mohd,"Stimultaneous determination of preservatives(benzoic acid scorbic acid methyl paraben and propyl paraben) in food stuffs using HPLC", J. chromate. Phy. A., 1073(1-2), 393-397 (2005).

9- السامرائي, شذى يونس يحيى, "تقدير عقار الكلونازيبام وبعض مركبات البارابين بأستخدام طرائق كهروكيميائية وطيفية" , إطروحة دكتوراة , جامعة تكريت , كلية العلوم , ص 61 – 72 (2015).

10-A. Myint, Q. Zhang and L. Liu and H. C. "Flow injection – chemiluminescence determination of paraben preservative in food Safety", J. Anal. Chem. Acta, 517(1-2), 119-124 (2004).

11-Q. Zhang, MeiLian, L. Liu and H. Cui,"High-performance Liquid chromatographic assay of parabens in Wash- off Cosmetic products and food using chemiluminesscence detection", J. Anal. Chem. Acta, 537 (1-2), 31-39 (2005).

التقدير الطيفي للمثيل بارابين في المستحضرات الدوائية بتفاعلات الأزدواج التأكسدي

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الملخص

تم تطوير طريقة طيفية حساسة ودقيقة لتقدير المثيل بارابين بواسطة الأقتران التأكسدي مع 4,2– ثنائي نايتروفنيل هيدرازين بوجود العامل المؤكسد ن– بروموسكسنيمايد في وسط قاعدي وقد تم قياس الأمتصاصية عند طول موجي (600) نانوميتر. تتبع الطريقة قانون بير في مدى من التراكيز (2 –20 مايكروغرام/مل), وقد بلغت الأمتصاصية المولارية (6253) لتر/مول. سم$^{-1}$, وقيمة دلالة ساندل (0.02433) مايكروغرام/سم^{2}, وحد الكشف (0.34) مايكروغرام/مل, وقيمة معامل الأرتباط (0.9947), وأسترجاعية (98.96 -103 %), وقد تم تطبيق الطريقة المقترحة بنجاح لتحديد المواد الحافظة في المستحضرات الصيدلانية وكذلك في مستحضرات التجميل.

الكلمات الدالة: الطريقة الطيفية , مثيل بارابين.