Eng. & Tech. Journal, Vol.30, No.13, 2012

Separation and Determination of Preservative Methylparaben in Insulin Preparations Using Gas Chromatography

Suhair Mohammad Yaseen
Electrical & Electronics Techniques Collage, Foundation of Technical Edu. / Baghdad
Email:SuhairYaseen@yahoo.com
Alberteen E. Habboush
Science College, University of Baghdad/ Baghdad

Received on: 19/4/2011 & Accepted on: 5/4/2012

ABSTRACT

In this search an improved gas chromatographic method was used for identification and typically quantitated for methylparaben(mp) which is used as preservative in insulin preparations.it was involved the optimal conditions for extraction mp from aqueous samples with ethyl acetate ,chloroform ,and toluene as extractants,then analyzed by gas chromatography(GC).

Effects of each of extractants polarity, aqueous samples pH, phases (organic to aqueous) ratio, mixing time, polarity of stationary phases, and column temperature on efficiency of separation and determination of mp. were studied in this work.

Synthetic sample which contained the same contents as a life sample was tested to omit interferings of other drug additives on analysis results.

The extraction of mp. was (90-91)% with one batch of ethyl acetate and (99-100)%with two batches of ethyl acetate at pH medium(1.5-2).also the non-polar stationary phase(OV-101) is the best choice for determining mp by GC.

The concentration of mp. in insulin preparations was within the acceptable range(1.003mg/ml).

The relative error (R.E) was ± (0,39-1.49)% and relative standard deviation(R.S.D) was (1.21)% for life samples.

Keywords: GC, methylparaben, extraction, preservatives, OV-101.

فصل وتقدير المثيل بارابين المركب الحافظ لمستحضرات الانسولين باستخدام تقنية كروماتوغرافيا الغاز

الخلاصة

في هذا البحث ،أستخدمت طريقة كروماتوغرافيا الغاز المطورة لتقدير مركب المثيل بارابين المستخدم لحفظ بعض مستحضرات الانسولين .وتضمنت أيجاد الظروف المثلى لاستخلاص هذا المركب من محلوله المائي بأستخدام خلات الاثيل،الكلوروفورم والتولوين وبعد ذلك يجرى تقدير المركب بأستخدام كروماتوغرافيا الغاز.

تمت دراسة تأثير كل من قطبية المستخلصات ،حامضية النموذج المـــائي ،نســـبة الاطـــوار (العضوية :المائية)،وقت المزج ،قطبية الاطوار الثابتة ودرجة التحليل المناسبة للعمود على كفاءة فصل وتقدير مركب المثيل بارابين .

https://doi.org/10.30684/etj.30.13.9
2412-0758/University of Technology-Iraq, Baghdad, Iraq

من أجل التخلص من تأثير المضافات الاخرى في مستحضرات الانسولين على كفاءة الفصل والتقدير فقد تم تحضير نموذج تركيبي مماثل بطبيعة المكونات وتراكيزها للنموذج الحي .

حقق أستخلاص المثيل بارابين مامقداره (90-91)% بدفعة أستخلاص واحدة من خلات الاثيل من الطور المائي بحامضية وسط 1.5-2 ومامقداره (99-100)% بدفعتي أستخلاص بأستخدام نفس المستخلص وبنفس الظروف .

كذلك فأن الطور الثابت اللاقطبي المتمثل بعمود البولي سيلوكسين (OV-101) يعتبر الخيار الامثل لتحليل المثيل بارابين بتقنية كروماتوغرافيا الغاز.

وقد وجد أن تركيز المثيل بارابين ضمن الحدود المقبولة (1.003ملغم /مل).

الخطأ النسبي لهذه الطريقة مقداره (0.39-1.49)% ،وأنحراف قياسي نسبي مقداره (1.21)% للنماذج الحية.

INTRODUCTION

Phenolic compounds with one hydroxyl group and one substitute in the benzene ring are used as preservatives [1,2]. These compounds were investigated on two polar and non-polar stationary phases in gas chromatographic techniques by Grzybowski and etal [3].

Parabens (methyl, ethyl, propyl and butyl p-hydroxy p-hydroxybenzoate) are good preservatives for different kinds of pharmaceutical preparations, cosmetics and food because of their broad antimicrobial spectrum with relatively low toxicity and good stability [4]. Rebbeck and etal developed an accurate solid-phase extraction(SPE)coupled with high performance liquid chromatography(HPLC)method for quantification of mp and propylparaben (pp.)and other researchers did [5,6,7,8,9]. Bajardi and Giannole reported analysis of these compounds by using gas chromatography after reacting with triflouroacetate [10]. Rapid separation of mp. has been achieved by thin layer chromatography[11].

EXPERIMENTAL APPARATUS

Gas Chromatography- Varian, Vista 6000 with flame ionization detector

Chromatographic Condition

Nitrogen was used as carrier gas at 30 cm^3/min. volumetric speed. Detector temperature should be (30-50)Cº higher than column temperature which should be more than injection room temperature by (30-50)Cº. All these conditions are necessary to ensure complete vaporization of samples. Attenuation between 2-8 to get the best peaks, with Varian linear recorder and (OV-17, OV- 225, OV -101) columns.

Materials

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

Insulin with methylparaben was commercially available in disposable vial (volume 10 ml) from NOVO NORDISK, Denmark and Lilly Company France.

Preparation Standards Curves

Standard solution of (60mg/ml) of mp. was prepared by dissolving suitable weight of mp. in ethanol.

1- 5%, OV-101 column at 200 Cº and 3cm/min as Chart speed.

By injection (0.1, 0.2, 0.3, 0.4, 0.5, 0.8)µl of standard solution (60 mg/ml), and the results are shown in figure(1) .

2- 10%, OV-101 column at 220 Cº and 1cm/min as Chart speed.
Injection volumes (0.1, 0.2, 0.3, 0.4,……..0.9)µl of standard solution (60 mg/ml), and the results are shown in figure(2) .

3- 3%, OV-17 column at 240 Cº and 3cm/min as Chart speed.
Injection volumes (0.05, 0.1, 0.2, 0.3,……..0.8)µl of standard solution (60 mg/ml), and the results are shown in figure(4).

4- 5%, OV-225 column at 235 Cº and 0.5cm/min as Chart speed.
Injection volumes (0.8-3)µl of standard solution (60 mg/ml), and the results are shown in figure (3).

SYNTHETIC INSULIN SAMPLE

It consists of [12]:

1- Methylparaben(1 mg\ml).
2- Sodium acetate (1.7 mg\ml).
3- Zinc chloride (2.5 mg\ml).
4- Di basic sodium meta phosphate (2.5 mg\ml).

PREPARATION OF SAMPLE

3ml of synthetic or life sample was taken and justified its pH to(1.5-2)by concentrated hydrochloric acid ,then 2ml of distillated water was added to above aqueous solution and mixed for five minutes with 1ml of ethyl acetate in separation funnel.

RESULTS AND DISCUSSION

Depending upon principle which says "like dissolve like it", extraction percentage of methylparben from its medium was increased with increasing polarity of organic extractants as in table (1):

Ethyl acetate >chloroform>toluene [13].

Extraction in acidic medium (pH=1.5-2) was better than basic medium as in table (1), because basicity of aqueous medium is converted methylparaben to it's salt which is more soluble in aqueous solution [14, 15].

Ratio of organic to aqueous phase was 1:5 or 1:10. It was made extraction effective.

Also, there is no effect of other chemical compounds in insulin preparation on extraction [16].

As shown in table (2),there was no noticeable different in extraction ratio with different mixing time.

In the studying gas chromatographic behavior of methylparaben,the high polarity of OV-275 (dicyano allyl poly siloxane) as stationary phase in gas chromatography technique was leaded to long retention time (R_t) of methylparaben because of strong interaction between this compound and stationary phase.

Other stationary phases which have low polarity in comparison with OV-275 such as OV-225 (cyanopropyl methyl polysiloxane) or OV-17 (phenyl methyl polysiloxane) were resulted good resolution of methylparaben, but tailing in peaks as shown in figures(5,6).

Using of non-polar stationary phase which represented by OV-101 (Dimethyl polysiloxane) was given sharp symmetrical peaks of methylparaben as in figures(7,8).The last stationary phase was depended in its work of compound analysis on vapor pressure of methylparaben without interaction with it as in figure (9).

The concentration of mp in insulin preparations was within the acceptable range (1.003mg/ml) as shown in table (8, 9).

CONCLUSIONS

A simple and sensitive method was developed for the analysis of preservative methylparaben in insulin preparations by extraction with ethyl acetate at pH=1.5-2,then GC,equipped with 10%OV-101 column and FID detector. This method offers the advantages of higher selectivity of MP, fewer interferings, and shorter analysis time. The method can be used for routine analysis of preservative MP in different pharmaceutical preparations.

REFERENCES

[1]Kibbe A.H.,Hand book of Pharmaceutical Excipients,3th ,pp.340,450,471,2000.
[2]Lin Y.H,Chou S.S.,Shgu Y.T.,J.Chromatogr.Sci., vol.345,p.38,2000.
[3]Grzybowski J.,Nasal A.,Radecki A.,J.Chromatogr.,vol.196,pp 217-223,1980.
[4]Food and Chemical Toxicology, vol.40, Issue 10, pp1335-1373, October 2002.
[5]Seong Ho Kang and Hasuck Kim,J.Pharmaceutical and Biomedical Analysis ,Vol.15,Issuses 9-10,PP1359-1364,June 1997.
[6]Rebbeck C.,Hammond R,Wong J,Nair L,Raghavan N,Hepler D,Drug Dev Ind Pharm., 32(9);1095-102, oct 2006.
[7]A.Abdollahpour,M.Forouhi,M.Shamsipurn,Y.Yamini,J.Iran.Chem.Soc.,Vol.7,No.2, PP.516-520,Jun.2010.
[8]Hajkova R.,Solich P.,Anal. Chim. Acta.,vol.91,p 467,2002.
[9]USP 30, United States Pharmacopeial Convention Inc., p.136, 2007.
[10][Bajardi M.,Ginnole L.,Chem. Abs.97,143207m,1982.
[11]Afshin Mirzaie,Ahmad Jamshidi,Analytical Chemistry,vol.20,no.2,April 2002.
[12]British pharmacopeia , vol. II ,p.804-809 ,1988.
[13]Burini G., J.Chromatogr.,A 213,664,1994.
[14]Edwin S. Gould, Mechanism and Structure in organic chemistry, 1959.
[15]Hajkova R.,Solich P.,Pospisilova M.,Anal.Chem.Acta,91,467,2002.
[16]Kibbe A.H.,Handbook of Pharmaceutical Excipients,3th ed.,pp.450,471,2000.

Table (1) Extraction of methylparaben at concentration 3mg/ml from acidic and basic media, using different solvents.

Solvent (extraction)	pH of aqueous phase	Phase ratio org:aq.	Extraction%
Chloroform	1.5-2.0	1:5	60.17
Chloroform	1.5-2.0	1:10	58.00
Chloroform	13-14	1:5	25.76-28.17
Chloroform	13-14	1:10	24.21-28.00
Toluene	1.5-2.0	1:5	20.00
Toluene	1.5-2.0	1:10	18.00
Toluene	13-14	1:5	0
Ethyl acetate	1.5-2.0	1:5	90.34-91.13
Ethyl acetate	1.5-2.0	1:10	90.38-91.13
Ethyl acetate	13-14	1:5	89.00-90.41

Table (2) extraction of methylparaben at concentration 3mg/ml from acidic medium at different mixing time.

Mixing time min.	% extraction chloroform	% extraction ethyl acetate
4	58.64	89.78
5	59.00	90.34
10	60.17	90.31

Table (3). Analysis of synthetic sample of insulin by using 5% OV-225 column with 0.76% R.S.D.

Sample no.	Sample vol., µl	Area under peak, cm^2	methyl paraben Concen.mg/ml	C.E	R.E
1	2	0.525	0.967	-0.033	3.413
2	2	0.536	1.002	0.002	0.199
3	3	0.700	0.998	-0.002	0.2
4	4	0.875	1.011	0.011	1.088
5	5	1.030	0.996	-0.004	0.40

Table (4) Analysis of synthetic sample by using 3% OV-17 column with 1.657 R.S.D. %

Sample no.	Sample vol., µl	Area under peak, cm^2	methyl paraben concen. mg/ml	Constant Error	RelativeError
1	9	0.11	1.04	0.014	1.38
2	9.6	0.15	0.996	-0.004	0.40
3	10	0.192	1.001	0.001	0.099
4	10	0.196	1.005	0.005	0.497
			Av.=1.004		

Table (5) Analysis of insulin preparation (ultratard) by using 3% OV-17 column with 1.017 R.D.S%

Sample no.	Sample vol., µl	Area under peak, cm^2	methyl paraben Conc.mg/ml	C.E	R.E
1	2.4	0.599	0.992	-0.008	0.806
2	3	0,693	0.983	-0.017	1.73
3	4	0.855	0.982	-0.018	1.83
4	4	0.864	0.995	-0.005	0.05
5	5	1.039	1.007	0.007	0.695

Table (6) Analysis of synthetic sample by 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 (7) Analysis of insulin preparation (monotard) by 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	0.410	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 (8) Analysis of insulin preparation (humulin) by using 10% OV-101 column with 2.636 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 (9)Analysis of synthetic sample by 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		

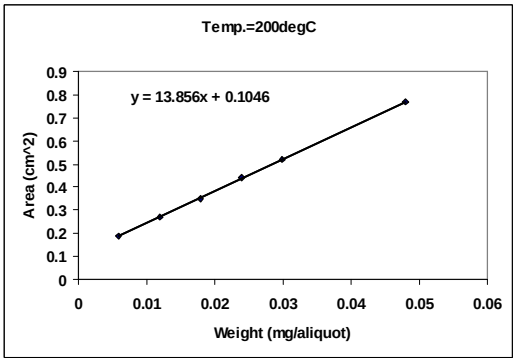

Figure (1) standard calibration curve for methylparaben on 5% , 0V-101 at 200 C°

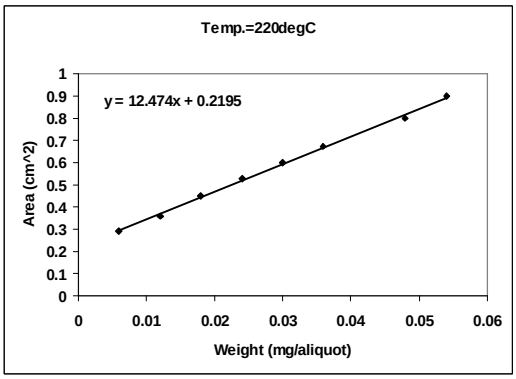

Figure (2) standard calibration curve for methylparaben on 10% , 0V-101 at 220 C°.

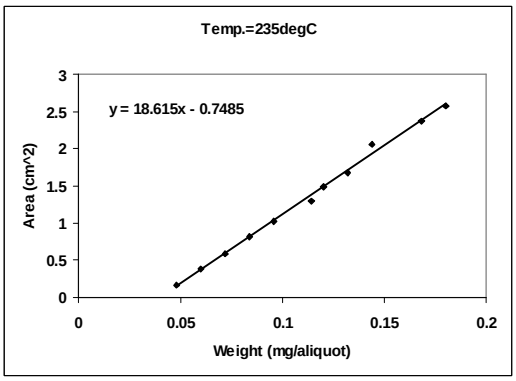

Figure (3) standard calibration curve for methylparaben on 5% , 0V-225 at 200 C°.

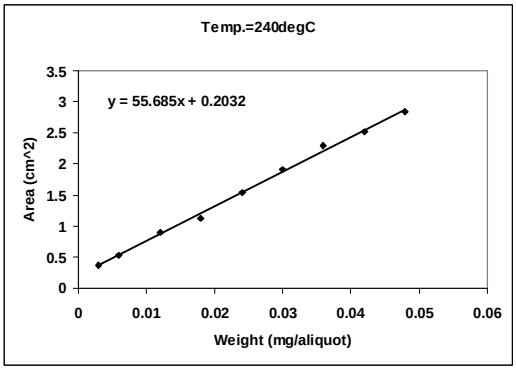

Figure (4) standard calibration curve for methyl paraben on 3% , 0V-17 at 240 C^{o}

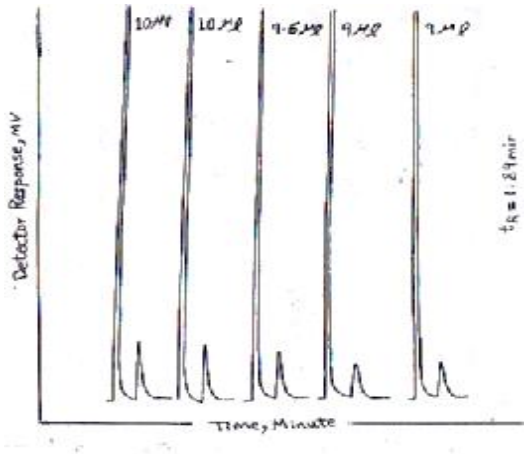

Figure (5) Chromatograms of methylparaben extracted in two batches by using acetate from Synthetic sample on 5%, OV-225 at 235C^{O}.

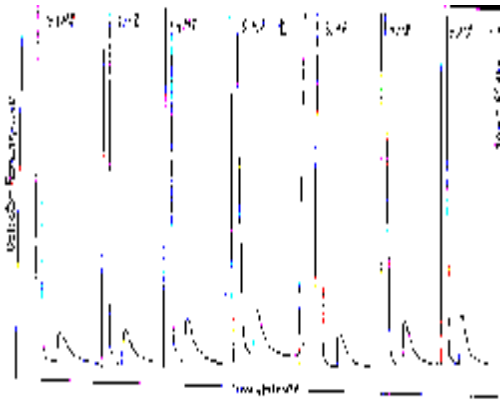

Figure (6) Chromatograms of methylparaben extracted in two batches by using Ethyl acetate from (a)synthetic sample, (b) an insulin injection (ultratard), on 3%, OV-17 at240 C^{O}

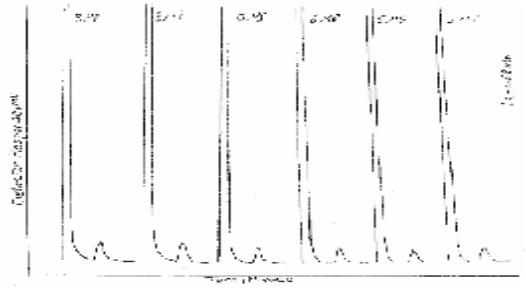

Figure (7) Chromatograms of methyl paraben extracted in one batches from synthetic sample by using Ethyl acetate on 5%, OV-101 at 200 C^{O}.

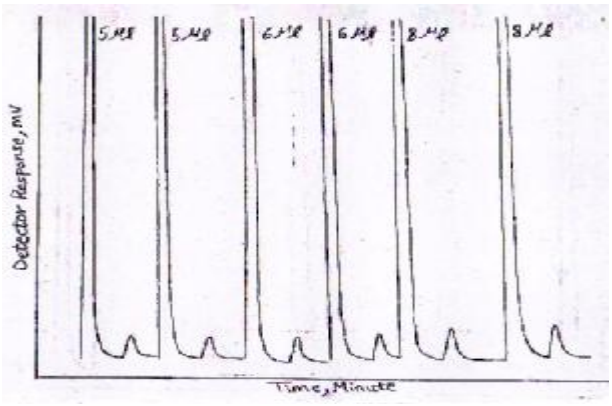

Figure (8) Chromatograms of methyl paraben extracted in one batches from an insulin injection (monotard) by using Ethyl acetate on 5%, OV-101 at 200C^{o}

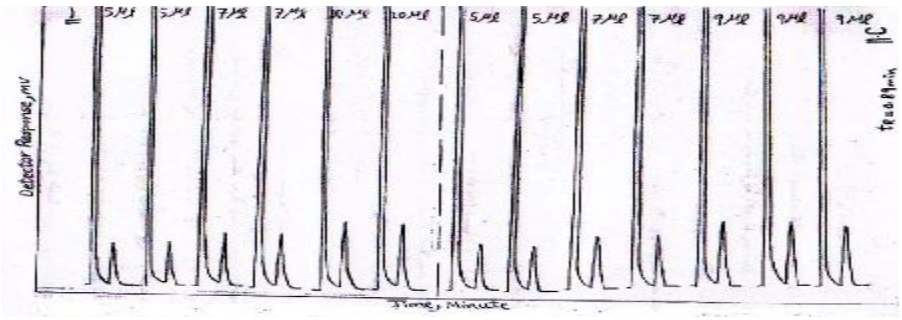

Figure (9) Chromatograms of methyl paraben extracted in two batches by using Ethyl acetate from an insulin injection (humulin) (a) from synthetic sample (b) on 10%, OV-101 at 220C^{o}