Bull. Pharm. Sci., Assiut University,
Vol. 16, Part 1, PP 1-11

ACCELERATED STABILITY TESTING OF IBUPROFEN-EUDRAGIT RSPM SUSTAINED RELEASE TABLETS USING ^{1}H-NMR ,HPLC AND TLC

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ABSTRACT

Ibuprofen-Eudragit RSPM sustained release tablets, already have been formulated, were subjected to accelerated stability testing for 6 months at 25, 37 and 45°C.The tablets were stored in firmly closed glass bottles in order to eliminate the effect of humidity, a condition simulating the actual shelf storage.

The drug content of the stored tablets was evaluated using ^{1}H-NMR, HPLC and TLC techniques in order to investigate the effect of temperature on ibuprofen stability.

A small reduction (2-4 % w/w) in the drug content was detected in the tablets stored at 37 and 45°C for 6 months using ^{1}H-NMR and HPLC techniques.The decompositiom of ibuprofen in the preformulated tablets was found to be first order reaction kinetics .

It was concluded that the expiry date of ibuprofen in the formulated sustained release tablets is up to 3 years. Thus this formulation could be considered as a convenient pharmaceutical formulation of ibuprofen.

INTRODUCTION

The classical method of determining the shelf life of a pharmaceutical preparation is to store it under conditions similar to those of the normal conditions and to study its stability for the expected period in the market[1].

The USP defines room temperature as the temperature prevailing in the working area and controlled room temperature as the temperature between 15 and 30°C. It is usual to use data from long term stability testing conducted at about 25°C and short term stability testing at higher temperatures to support an expiration dating period with a storage condition between 15 and 30°C[2]. High temperature accelerated stability studies were employed to predict the shelf life of the drug in a considerably shorter time.The application of such kinetic studies is used in food and pharmaceutical industries[3]. Elevated and low temperatures, also cycling of temperature between elevated and low may be used[4]. The 90 % of the drug remaining at 25°C, the shelf life, is then determined from Arrhenius plot[5].

HPLC was used as a method for evaluation of ibuprofen for accelerated stability testing in bulk drug and tablets and dosage uniformity testing[6]. This technique has been also used for determining ibuprofen in human plasma[7-9].

^{1}H-NMR spectroscopic method was developed for the assay of haloperidol[10] and phenylbutazone[11] in commerical tablets.

In the present work, the stability study at room temperature (25°C) and accelerated stability at 37 and 45°C have been carried out on the previously optimized ibuprofen-Eudragit RSPM sustained release tablets[12]. The drug content was evaluated using HPLC, ^{1}H- NMR and TLC techniques.

EXPERIMENTAL

Materials:

P-phenylphenol was used as internal standard for HPLC and ^1H - NMR (Eetman organic chemicals, Rochester). Dimethyl sulphoxide (DMSO-d_6) deuterated was used as ^1H-NMR solvent (Merk Sharp and Dohme, Ltd.). Tetramethylsilane (TMS) was used as a reference standard for ^1H-NMR analysis (Aldrich chemical Co.). Acetonitrile, acetic acid and chloroform were also used and were of spectrophotometric or analytical grade. Flourscent silica gel G plates (0.2-0.5 mm). (Merk Sharp and Dohme, Ltd).

Equipment:

HPLC apparatus with variable wave length detector, (750 Waters Associates, Millford Mass, USA). Octadecylsilane column (Dupont instrument, Willimington, Deleware, USA). Filter membrane (Millipore membrane filtering apparatus, Millipore Corporation).

200 MHZ ^1H-NMR (Nuclear magnetic resonance apparatus, Brucker, Ac 200).

Methods:

The previously prepared optimized[12] formula of ibuprofen-Eudragit RSPM sustained release tablets granulated with 15% w/v Eudragit RSPM and containing 23 % w/w Avicel PH 102 and 1 % w/w magnesium stearate were subjected to stability testing at 25, 37 and 45°C for 6 months by storing them in firmly closed colourless glass bottles just after compression to eliminate the effect of light and humidity.The drug content of the stored tablets was determined at specific time intervals as follows:

1.HPLC evaluation:

The liquid chromatographic system consisted of pump, injector valve with 20 µl sample loop, wavelength UV detector (254 nm) and integrator.A stainless steel column, 150 x 4.5 mm packed with 5-µm octadecylsilane was used. Acetonitrile-0.25 M acetic acid (75:25 v/v) was used as the mobile phase which was filtered and deaerated prior to use. The flow rate was 1.2 ml/min. at room temperature.

Standard solution preparation for HPLC:

Five mg of p-phenylphenol, the internal standard, was dissolved in 100 ml of the mobile phase. Accurately weighed 5 mg of standard ibuprofen was transferred to 10 ml volumetric flask to which 1 ml of the internal standard was added. The mobile phase was completed to volume and the solution was mixed and filtered.

Single tablet assay for dosage uniformity by HPLC:

One tablet was placed in 250 ml volumetric flask containing 50 ml of 0.25 M acetic acid. The flask was shaken until the tablet disintegrated. Acetonitrile, 150 ml, was added followed by the addition of the mobile phase to volume.Ten ml of the mixture were filtered, discarding the first 2 ml of the filtrate. An aliquot, equivalent to 5 mg of ibuprofen, was transferred to a 10 ml volumetric flask containing 1 ml of the internal standard and diluted to volume with the mobile phase.

HPLC purity:

A test solution containing 500 µg of the drug/ml of the mobile phase was prepared. This solution and a mobile phase blank were chromatographed using increased detector senstivity to insure the detection of any peak.

Accelerated stress studies:

Accelerated degradation of ibuprofen was accomplished by several methods[12]: (1) ibuprofen (50 mg) was heated at its melting point

(76-90°C) for one hour, (2)ibuprofen (50 mg) was dispersed in 5 ml of N 1 HCl and kept at 100°C for 3 hours, and (3) ibuprofen (50 mg) was dispersed in 5 ml of N 1 NaoH and kept at 100°C for 3 hours. After 3 hours, the acid and base samples were neutralized and diluted to 25 ml with methanol. The sample heated at melting point was completed to 25 ml with methanol. HPLC and TLC evaluations were carried out.

TLC was carried out by spotting the equivalent of 100 μg ibuprofen on silica gel G flourescent plates and developing them in tanks lined with adsorbent papers in the following mobile phase, ether; 1-butanol: benzene: methanol (85:6:8:1). They were visualized under UV light.

Assay procedure:

Five replicate injections of the standard solution were done. The response ratio of ibuprofen and p-phenylphenol peak areas was determined. When the reproducibility was such that the relative standard deviation of the five response ratios was not greater than 3%, duplicate injections of the sample were done. The average peak area ratio of ibuprofen relative to p-phenylphenol was determined.

HPLC calculation:

The quantity of ibuprofen (mg) in the portion of the drug sample taken was (Ru/Rs) x Ws where Ru and Rs were the peak area ratios obtained from the sample and the standard solution respectively,and Ws was the weight (mg) of ibuprofen taken. The quantity of ibuprofen (mg) per tablet was: C(Ru/Rs) x VD/N, where C was the concentration of the standard solution (mg/ml), V was the volume (ml) of the tablet sample solution, D was the dilution factor and N was the number of the tablets taken.

2. ^{1}H-NMR evaluation:

The procedure was carried out as follows: Ten tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 20-30 mg ibuprofen was transferred to a 15 ml graduated glass stoppered centrifuge tube. About 20-30 mg of p-phenylphenol, accurately weighed, was added. The tube was filled with DMSO-d_6 to the 2 ml mark.With the aid of a vortex mixer, the solution was stirred, keeping the solution down from contacting the stopper and centrifuged. About 0.5 ml of the supernatant was transferred to an analytical NMR sample tube that contained 2 drops of tetramethylsilane (TMS). The spectrum was recorded using 200 MHZ ^{1}H-NMR apparatus. The spinning rate used would not produce any interferring spinning side bands in the spectral regions of 7.1-7.7 and 6.0-6.8 ppm. The resonance signals for ibuprofen appeared as two doublets centered at 7.12 ppm. The multiplet and the doublet for p-phenylphenol, the internal standard, appeared at 7.5 and 6.8 ppm were integrated. A minimum of five integrations was done and the results were averaged. The average integral values were obtained and the quantity of ibuprofen was calculated as follows:

Ibuprofen (mg) per tablet = (Au/As) x (E Wu/E Ws) x C x (T/W)

Where:

Au = the average integral value of the resonance signal of ibuprofen centered at 7.12 ppm

As = the average integral value of the resonance signal of p-phenyl phenol at 7.5 ppm

E Wu = the mdecular weight of ibuprofen divided by the number of absorbing protons, i.e., (206.3)/4 = 51.58

E Ws = the mdecular weight of p-phenylphenol divided by the number of absorbing protons.i.e., 170/9 = 18.89

C = the weight in mg of p-phenyl phenol taken for the analysis

T = the average tablet weight, mg

W = the weight of the sample taken for the analysis, mg

RESULTS AND DISCUSSION

The formulated tablets were stored for 3 and 6 months at 25, 37 and 45°C in firmly stoppered coloured glass bottles to eliminate the effect of light and humidity on the stability of the stored tablets, a condition simulating the actual shelf storage.

A rapid and simple stability-indicating assay procedure for ibuprofen alone and in tablets was developed using HPLC to test stability as well as dosage uniformity. In this procedure, the mobile phase used was acidified with acetic acid to pH 3.7 to suppress ibuprofen ionization and reduce peak tailing[6]. Ibuprofen and the internal standard (p-phenylphenol) were well resolved. The retention times were 2.136 and 1.92 minutes respectively. The peak area ratio response was shown to be linear throughout ibuprofen concentration range from 0.075 to 2 ug/ml, with a correlation coefficient of 0.999. Precision was demonstrated by a standard deviation of 0.5 % for 10 replicate standard injections. No detectable interference from impurities was detected under the chromatographic procedure described.

The accuracy and validity of the method were determined by the following experiments : (a) Reproducibility,which is the expression of the precision of HPLC method which is practically carried out by injecting 5 mg sample preparations using the same apparatus and the same conditions. Fig.1 shows that the HPLC procedure adopted for ibuprofen determination was reproducible, (b) Reproducibility, which is the expression of accuracy of HPLC method. This can be carried out by adopting the last mentioned procedure. Fig.1 shows that the adopted HPLC procedure to be accurate, (c) Recovery of ibuprofen, 5 weights of placebo of each pharmaceutical formulation to which known quantities of ibuprofen have been added were assayed. Figs. (2-4) demonstrate that the formulated tablet excipients did not interfere with ibuprofen assay, and (d) linearity, which is the relationship between the quantity of ibuprofen which constitutes the dose and the resulting response (peak area), this relation was linear, Fig.5. From the last obtained results, it could be concluded that the adopted HPLC procedure was accurate and precise in determining ibuprofen in the prepared sustained release tablets.

Accelerated stress study was conducted to assess the efficacy of the assay procedure[12]. The sample subjected to acid hydrolysis turned yellow during the first 2 hours. The chromatogram of this sample showed 2 extra peaks of retention times of 1.9 and 2.11 minutes respectively.

HPLC simplifies the dosage uniformity test. The recovery of ibuprofen from each mixture containing the tablet excipients and ibuprofen was carried out through the described HPLC procedure.The average recovery was 100.5 %, Table 1.

HPLC assay procedure was performed on ten single tablets stored at 25, 37 and 45°C in tightly closed coloured glass stoppered bottles. The analysis showed that the average percentage of declared potency

ranged from 96 to 100%. The accuracy of the assay represented by these results is illustrated in Table 2. In conclusion, the HPLC procedure is accurate, precise and practical for estimating ibuprofen in the stored tablets.

The stressed samples were qualitatively examined by TLC using flourescent silica gel G and ether: 1-butanol: benzene: methanol (85:6:8:1) as a developing system. TLC examination revealed the presence of one spot with R_f value of 0.83 which equals to that for reference drug. The acid hydrolyzed sample showed two spots. These results are in qualitative agreement with HPLC findings, Fig.6. There was no colour change for the sample subjected for base hydrolysis as it showed a faint original spot on TLC.

TLC examination of ibuprofen tablets stored at 37 and 45°C for 6 months revealed the presence of one spot of 0.83 similar to that of reference ibuprofen.

^{1}H-NMR was used for ibuprofen quantification in the stored tablets. Ibuprofen exhibited a well resolved signal further downfield, which was found to be satisfactory for quantitative work. P-phenylphenol, the internal standard, was completely soluble in DMSO-d_6 and its nine biphenyl protons produced a strong resonance signal as the convenient downfield of 7.5 and 6.8 ppm. The 200 MHZ ^{1}H-NMR spectrum of a mixture of ibuprofen and p-phenyl phenol is shown in Fig.7. The chemical assignements and multiplicities of the resonance signals of ibuprofen are given in Table 3. The resonance signal of relevance to the assay corresponds to the two doublets at 7.2 and 7.1 ppm due to the phenyl protons of ibuprofen at positions (a, a`) and (b,b`) respectively. A multiplet appeared at upfield position (1.8 ppm) due to methine protons at position (d), while the three doublets appeared at positions 2.4, 1.35 and 0.85 ppm and the quartet appeared at position 3.65 ppm are due to the methyl group at position (h), the methylene group at position (c), the two methyl groups at (e, f) and the methine proton at position(g) respectively. Fig.8 shows the ^{1}H-NMR signals for ibuprofen in the tablets with the internal standard which is identical to Fig.7.

The quantity of ibuprofen present in the tablets was determined by integrating the two doublet resonance signal pattern centered at 7.2 and 7.1 ppm arised from the four aromatic protons at positions (a, a`) and (b, b`). The multiplet and doublet at 7.5 and 6.8 ppm respectively are due to the nine protons of the internal standard. In addition to their utility in the quantitative analysis of ibuprofen in the tablets, the proton resonance characteristics of this molecule could be used for identification.

The analysis of several known ibuprofen mixtures with p-phenyl phenol is summarized in Table 4 . The results showed that the method was both accurate and precise over the range of analyte to internal standard studied. The mean recovery value SD (100.3 % ± 0.77 %, n=5). The coefficient of variation (C.V) for this method was less than 1 %.

The assay of 300 mg unaged tablets of ibuprofen yielded the results presented in Tables (5) and Fig.(8). Sustained release tablets preparations spiked with known amounts of drug gave a mean recovery value SD (99.8 % ± 0.66%, n=5). The proposed method was also applied to evaluate ibuprofen in tablets stored for 6 months at 37-C, Table (6). Comparing the results in Table (5) with Table (6) shows that the drug content of the tablets stored for 6

months at 37°C is lower by 0.92% than those after direct preparation.

Table (7) illustrates the application of HPLC and ^{1}H-NMR methods for the analysis of dosage uniformity of ibuprofen in tablets at 25 and 37°C. The results obtained from both methods are nearly the same giving good evidence of the drug stability and the accuracy of both methods in support to each other.

In order to investigate the effect of temperature on the stability of ibuprofen using ^{1}H-NMR method, the results given in Tables (4-6) were analyzed. Therefore, the calculation of the stability reaction rate constants for ibuprofen in the stored tablets was done.

The highest correlation coefficients were fitted with the first order kinetics[13]. This was also confirmed since plotting of the logarithms of the remained percentage of ibuprofen versus time yields a straight line, Table (8) and Fig. (9). The reaction rate constant (k) and the time required for ibuprofen to drop to 90 % of its original concentration in the formulated tablets were calculated from the first order reaction kinetic equation, Table (8)[13].

A strong negative correlation between log concentration of ibuprofen remained and time was obtained as indicated by the high correlation coefficient (r), which confirmed the first order kinetics of ibuprofen decomposition in the sustained release formulated tablets.

It could be concluded that the optimized sustained release ibuprofen tablet formulation under investigation ,stored for 6 months at 45°C in firmly closed coloured glass bottles, kept 90 % of its original ibuprofen content, and hence it can remain stable for at least three years at room temperature[4].

Table (1): Recovery of Ibuprofen Synthetic Mixtures by HPLC Method.

Synthetic Mixture	Ibuprofen (mg)		Recovery (%)
	Added	Found	
1	200.5	201.0	100.3
2	310.0	309.8	99.6
3	298.4	299.0	100.2
4	300.0	305.0	101.7
5	404.0	405.5	100.8
		Average	100.50
		S.D ±	0.79

Table (2) : Effects of Temperature on Ibuprofen* Assay Results by HPLC Method.

Temperature	Storage Period (Months)	Percent of Labelled Found [a]
25°C	0	100.20
25°C	3	99.94
25°C	6	99.06
37°C	3	100.05
37°C	6	98.95
45°C	3	98.12
45°C	6	96.80

(a) mean results of five single-tablet assays.
(*) Ibuprofen (300 mg) tablets granulated with 15% W/W Eudragit RSPM.

Table (3): ^{1}H-NMR Spectral Assignments for Ibuprofen.

Proton Identification[a]	Chemical Shift ppm(δ)	Multiplicity
e,f	0.85	doublet
c	1.35	doublet
d	1.90-1.60	multiplet
h	2.4	doublet
g	3.65	quartet
a,a'	7.20	doublet
b,b'	7.10	doublet

(a) For location of protons see Fig.(g),DMSO-d_6 contained an impurity absorbing at 2.6 ppm, moisture absorbed at 3.35 ppm.

Table (4) : Analysis of Synthetic Mixtures by ^{1}H-NMR Spectroscopy.

Synthetic Mixture No.	p-phenyl -phenol	Ibuprofen		
		Added (mg)	Found (mg)	Recovery (%)
1	20.07	35.2	35.6	101.13
2	11.00	15.2	15.3	100.63
3	61.08	60.3	59.9	99.34
4	19.25	21.2	21.0	100.95
5	30.18	110.5	110.0	99.58
				100.33
			SD ±	0.77
			CV% ±	0.77

Table (5): Analysis of Ibuprofen Tablets by ^{1}H-NMR Spectroscopy Method After Direct Preparation.

Sample	Amount Declared (mg/tablet)	Amount Found (mg/tablet)	Amount Found % of Declared
1	300	300.5	100.17
2	300	298.0	99.33
3	300	299.5	99.83
4	300	302.0	100.67
5	300	297.0	99.00
			99.80
		SD ±	0.66
		CV% ±	0.66

Table (6): Analysis of Ibuprofen Tablets by ^{1}H-NMR Method After 6 Months at 37° ± 0.5°C.

Sample No.	Amount Declard (mg/tablet)	Amount Found (mg/tablet)	Amount Found % of Declared
1	300	296.15	98.72
2	300	300.20	100.07
3	300	295.81	98.60
4	300	292.00	97.33
5	300	299.10	99.66
			98.88
		SD ±	1.06
		CV% ±	1.07

Table(7): Ibuprofen tablet Analysis for Dosage Unformity by Different Methods.

Method of Analysis	Labelled (mg/tablet)	Conditions		% of Label Claim
		Temp.	Months	
HPLC	300	25°C	0	100.20
	300	37°C	6	98.95
^{1}H-NMR	300	25°C	0	99.80
	300	37°C	6	98.88

Table (8): Stability Reaction Rate Constant and $T_{0.9}$ of Ibuprofen - Eudragit RSPM Tablets Stored for 6 Months at Different Temperatures [25°, 37° and 45°C].

Parameter	Temperature		
	25°	37°	45°
Slop X 10^{-3}	-0.83	-0.917	-2.5
r	-0.959	-0.919	-0.993
K X 10^{-3}	-1.9	-2.1	-5.8
$T_{0.9}$ (m)	55	50	18

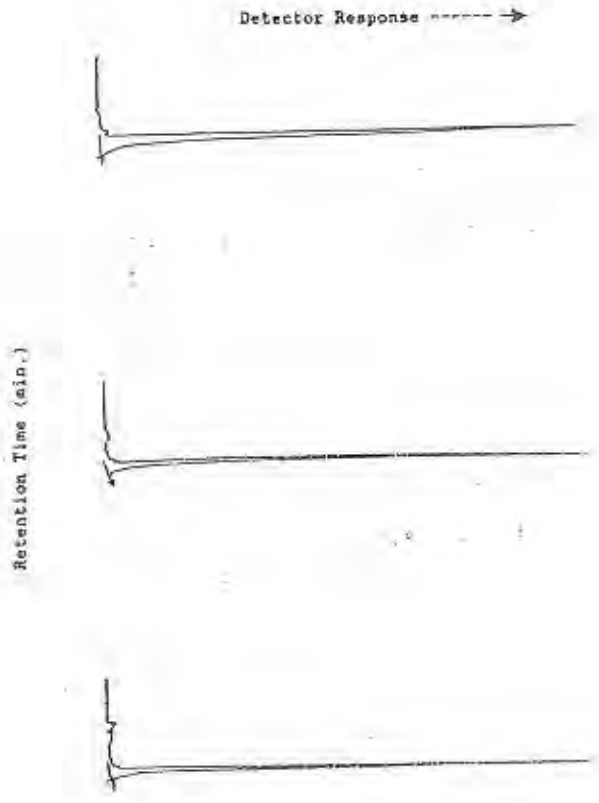

Fig.(1): Reproducibility of the Standard Dosage Form of Ibuprofen by HPLC Method.

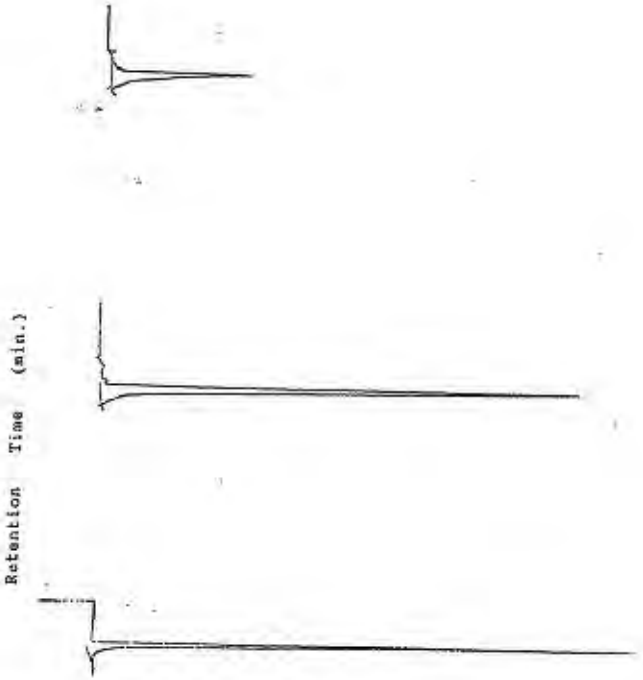

Fig.(5): Sample Chromatogram Showing the Relation Between the Concentrations and the Responses [Linearity].

Fig.(6): HPLC Chromatogram Showing Hydrolysis of Ibuprofen by (1 N HCL).

Fig.(7): Typical HPLC Chromatogram Obtained From the Excipients Without Ibuprofen.

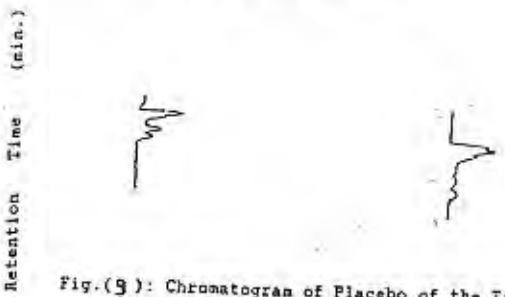

Fig.(8): Chromatogram of Placebo of the Tablet With Unspicked Ibuprofen.

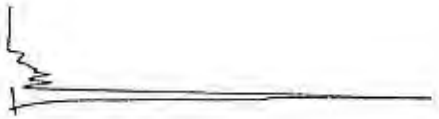

Fig.(9): HPLC of Ibuprofen Tablets.

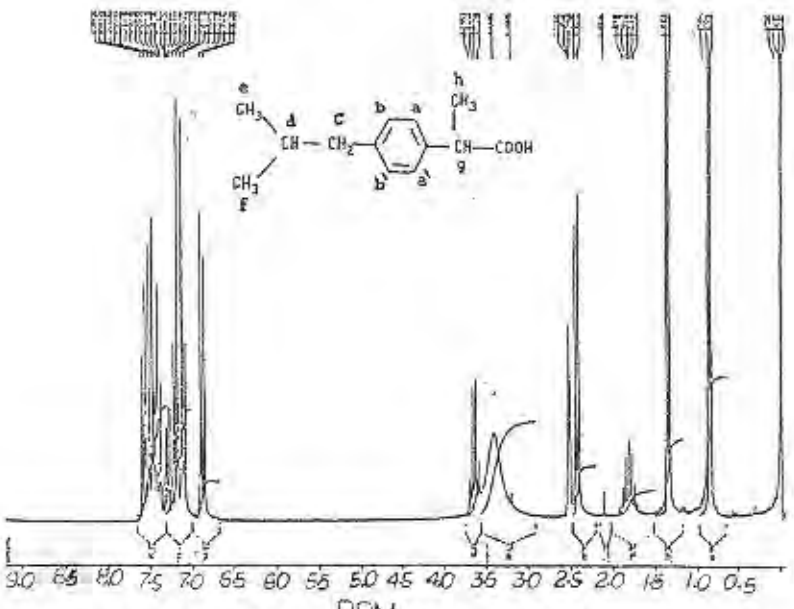

Fig.(7): 200 MHz ^{1}H-NMR Spectra of Ibuprofen and p-phenylphenol, the Internal Standard, in DMSO-d_6 Solution for Synthetic Mixtures.

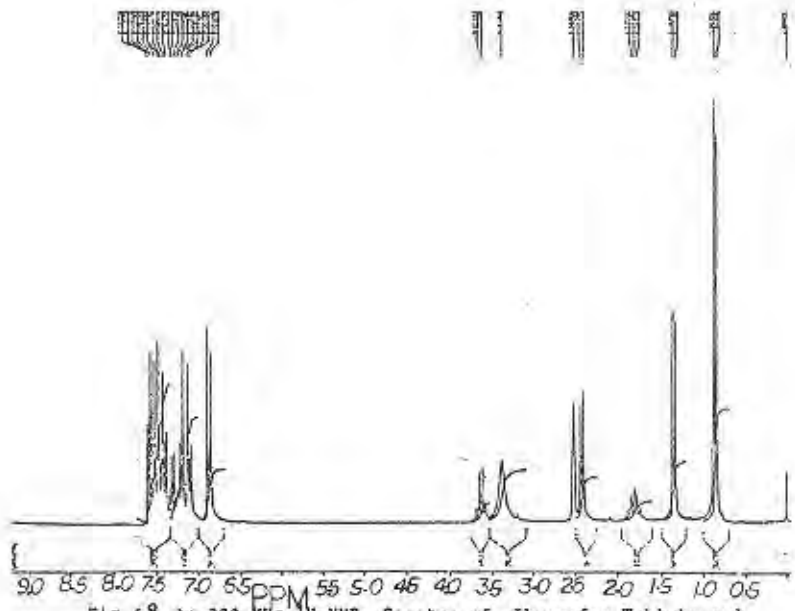

Fig.(8): 200 MHz ^{1}H-NMR Spectra of Ibuprofen Tablets and p-phenylphenol, the Internal Standard, in DMSO-d_6 Solution.

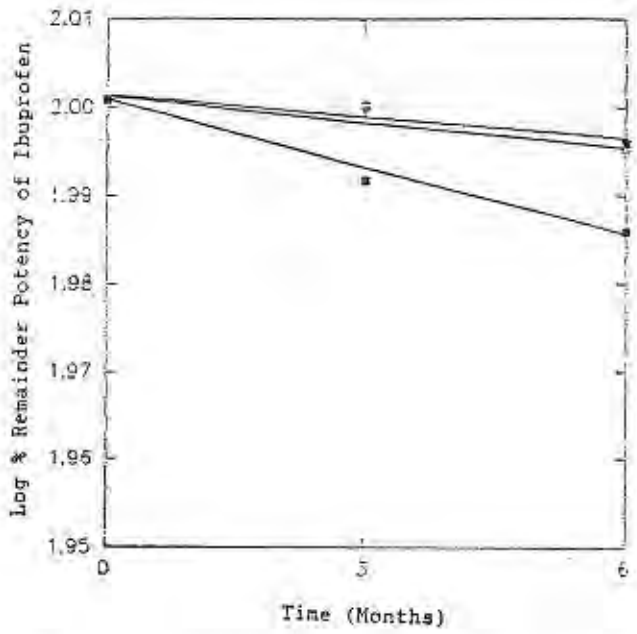

Fig.(9): Effect of Storage on the Stability of Ibuprofen from Its Tablets. using H-NMR.

Key, ▼ Tablets stored for 6 months at 25° C
○ Tablets stored for 6 months at 37° C.
■ Tablets stored for 6 months at 45° C.

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تقييم عقار الابوبروفين فى أقراص محضرة ممتدة المفعول فى دراسة ثبات سريع بواسطة الرنين النووى المغناطيسى وكروماتوجرافيا السوائل ذات الضغط المرتفع وكروماتوجرافيا الطبقة الرقيقة

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أقراص الابوبروفين - ايدراجيت *RSPM* طويلة المفعول والمحضرة - سابقا عرضت للثبات السريع عند درجات حرارة ٢٥ ، ٣٧ ، ٤٥ درجة مئوية وفى زجاجات ملونة ومقفلة تماما لمنع تأثير الرطوبة والضوء - لمدة ستة أشهر.

ولقد عين الابوبروفين فى الأقراص المخزنة كميا باستخدام الرنين النووى المغناطيسى وكروماتوجرافيا السوائل ذات الضغط المرتفع واستخدمت كروماتوجرافيا الطبقة الرقيقة لدراسة الثبات نوعيا.

ولقد ثبت أن الطرق المستعملة فى تعيين العقار كميا دقيقة وتعضد بعضها بعضا.

وقد لوحظ نقصا فى كمية العقار بالتخزين فى درجة حرارة ٣٧ ، ٤٥ درجة حرارة لمدة ستة أشهر ولقد وجد أن تحلل العقار يتبع معادلة من الدرجة الأولى.

ولقد وجد من تلك الدراسة أن الابوبروفين فى الاقراص المحضرة له تاريخ نهاية مفعول يمكن ان تمتد لاكثر من ثلاثة سنوات ولهذا تعتبر صياغة الابوبروفين فى تلك الاقراص صياغة يمكن أن تستعمل فى سوق الادوية ممتدة المفعول لهذا العقار.

Received in 2/6/1992 & Accepted in 20/2/1993