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Iraqi Pharmaceutical Formula for Clonazepam Oral Drop 2.5 mg/ ml to Treat Seizure Epilepsy in Infants and Children with its Stability Study.

Qusae faddel abas **zina medhat ebrahim** **hadil harith khalid** **amer khazal sahi**

Minisitry of Industry, Minerals opration for Research and Industrial Development IBN SINA

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Abstract

This work has been carried out to develop national drug product contains 2.5mg/ml clonazepam as oral drop; it is used for the treatment of epilepsy in infants and children.

Several formulations were prepared using oral drop base, flavor, buffer, sweeteners and preservatives. Selection of best formula relied solely on physic-chemical testing of samples.

Stability study was conducted on the product for six months at different temperatures to determine the expiration date and the best storage conditions.

From the study we obtained an oral drop of good clear solution. The expiry date calculated to be not less than 2 years.

Key words: Clonazepam, Oral Drop, Seizure Epilepsy.

تركيبة صيدلانية عراقية لمستحضر كلونازيبام قطرة فموية 2.5 ملغم/ مل لعلاج التشنجات العصبية والصرع عند الرضع والاطفال مع دراسة ثباتيتها.

قصي فاضل عباس زينة مدحت ابراهيم هديل حارث خالد عامر خزعل ساهي
مركز ابحاث ابن سينا/ هيئة البحث والتطوير الصناعي
وزارة الصناعة والمعادن

الخلاصة

نفذ هذا العمل لتطوير منتج دوائي وطني يحتوي على 2,5 ملغم /مل كلونازيبام على شكل قطرة فم والذي يستخدم لعلاج الصرع عند الرضع والأطفال. حضرت عدة تركيبات باستخدام قاعدة قطرة الفم، نكهة، بفر، محليات والمواد الحافظة اختيرت أفضل تركيبة بالاعتماد على الاختبارات الفيزيائية والكيميائية للتركيبات المحضرة.

أجريت دراسة الثباتية على التركيبة النهائية لمدة (6) أشهر عند درجات حرارة مختلفة لتحديد تاريخ انتهاءالصلاحية وأفضل ظروف التخزين.

من الدراسة حصلنا على محلول قطرة فموية جيدة الشفافية. تاريخ انتهاء الصلاحية حسب على ان لا يقل عن سنتان.

الكلمات المفتاحية: كلونازيبام، قطرة فموية، نوبة الصرع.

Introduction

Epilepsy is a chronic medical disorder or condition, usually resulting in unpredictable, unprovoked recurrent seizures that affect a variety of mental and physical functions. It is one of the most common neurological diseases, affecting more than 3 million people in the U.S. and about 50 million people worldwide. Epilepsy was one of the first brain disorders to be described. It was mentioned in ancient Babylon more than 3,000 years ago.Through the ages, the strange behavior caused by some seizures has led to the creation of numerous superstitions and prejudices.(3)

Effective pharmaceutical ingredients must be formulated in a suitable dosage form to enable the patient to get active and safe drug with good features. Clonazepam belongs to a group of medicines called benzodiazepines, it is used for the treatment of epilepsy in infants, children and adults, and they are thought to work by their action on brain chemicals.(1)

Clonazepam is a slightly yellowish, crystalline powder, practically insoluble in water, slightly soluble in alcohol and in methanol.(4)

The formula of clonazepam oral drop contains clonazepam 2.5mg/ml as an active ingredient and it is a generic drug that is not manufactured in the Iraqi factories, Therefore, the aim of study is to prepare an Iraqi formula for this dosage form with its stability study that is to be compatible with specifications of British pharmacopeia. This study is necessity and it's considered to be one of the important documents for the purposes of registration in the Iraqi ministry of health**.**

Materials and methods

Table (1): list of ingredients.

Item No.	Constituents	Quantity/ 100ml
1.	Clonazepam	0.25 gm
2.	Citric acid	0.001 gm
3.	Ethanol 96%	2.0 gm
4.	Glycerin	5.0 gm
5.	Methyl paraben	0.15 gm
6.	Propylparaben	0.05 gm
7.	Peach flavor	0.005 gm
8.	Propylene glycol	5.0 gm
9.	Sodium saccharin	0.2 gm
10.	Sodium citrate	0.002 gm
11.	Sorbitol	10 gm
12.	Deionized water	Add to 100 ml

The equipments used in this study are electronic balance, stainless steel mixer, pH meter and glass wears.

Procedure:

In suitable Pyrex beaker transfer the following materials, sodium saccharin, sodium citrate, citric acid and distilled water then heat at 70°C for 15 minutes with stirring. In another suitable beaker transfer the stated amount of propylene glycol and heat on water bath at 50°C then dissolve clonazepam with continuous stirring, In another suitable beaker transfer the following materials: methyl paraben, propyl paraben,ethanol 96%, glycerin, sorbitol, and peach flavor, then mix for 10 minutes, all the beakers contents were mixed with stirring for (15) minutes, then we check the pH with adjustment, since it should be between 4 –5.5, then we filled the product in a 20 ml amber glass bottle with dropper(12).

Method of analysis: HPLC method for analysis were followed according to the following: For each 1ml solution that contains 2.5mg clonazepam.

No.	Test	Specifications
1	Assay method	British pharmacopeia 2013 with modified.
2	Assay limit	95 – 105 %
3	pH limit	4 – 5.5
4	HPLC conditions : A. Colum : B. Detection : C. Flow rate : D. Solvent : E. Mobile phase :	 C18 15 cm×3.9 254 nm. 0.8 ml/min. acetonitrile 50% acetonitril and 50% water

Assay:

Standard:

Weigh accurately 50 mg of clonazepam standard and dissolve to 100 ml with acetonitrile, then dilute 1ml of this solution to 10 ml of acetonitrile, this will produce a concentration of 0.05 mg/ml clonazepam.

Test:

Take 1ml of the sample, complete volume up to 50ml with acetonitrile, mix well, and then filter, this solution has a concentration of 0.05 mg/ml clonazepam.

Procedure: separately inject equal volume about 10µl of the standard and test solution into the chromatograph, record the chromatograms and measure the area under the curve(2).

Calculation:

$$\% \text{ of clonazepam} = \frac{AUP.Test}{AUP.STD.} \times 100$$

AUP = Area under the peak

Pharmacopoeial specification of clonazepam oral drop:

1. Description: clear oily solution.
2. Color: faint yellow.
3. Composition: each 1ml drops contains 2.5mg clonazepam.
4. Assay method: B.P 2013 with modified.
5. Assay limit: 95– 105%.
6. Filling: 20 ml amber glass bottle with dropper.
7. pH limit: 4– 5.5.
8. Expire date: 2 years from date of manufacture.

Results and discussion:

In this study, different formulas of oral drop were prepared according to the specification of British Pharmacopoeia 2013 presented in (Table, 2) and (Table, 3) .

The prepared clonazepam drops was found to be compatable with the stated Pharmacopoeial specifications and the results of stability studies are presented in Table (4 , 5 and 6).

A clear oily solution oral drop of acceptable consistency was produced and the physicochemical properties.

The product showed a good stability at different temperatures 25, 40, 50, and 60 °C.

The product was chemically stable at all these temperatures.

The result of stability study showed that the clonazepam oral drop product has good stability.According to this study, the expiration date has been estimated to be not less than

2 years from the date of manufacturing at room temperature.

Table (2): Quantitative composition of clonazepam oral drop Formulations (% wt/vol).

Ingredients (%)	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
Clonazepam	0.25	0.25	0.25	0.25	0.25
Citric acid	0.001	0.0015	0.0015	0.0015	0.001
Ethanol 96%	2.0	*	*	*	*
Glycerin	5.0	6.0	7.0	8.0	9.0
Methyl paraben	0.15	0.15	0.15	0.15	0.15
Propylparaben	0.05	0.05	0.05	0.05	0.05
Peach flavor	0.005	0.005	0.005	0.005	0.005
Propylene glycol	5.0	10	10	15	20
Sodium saccharin	0.2	0.2	0.2	0.2	0.2
Sodium citrate	0.002	0.0025	0.003	0.0025	0.002
Sorbitol	10	12	13	14	15
Distilled water to produce	100	100	100	100	100

Where, *Denotes that ethanol 96% is not added.

Table (3): Physicochemical properties of formulations.

Formula	Assay	pH	Appearance	Clarity
Formula 1	99.5	4.95	faint yellow oily solution	Clear
Formula 2	97.8	4.95	faint yellow oily solution	Turbid
Formula 3	98.6	4.85	faint yellow oily solution	Turbid
Formula 4	98	4.85	faint yellow oily solution	Turbid
Formula 5	96.6	4.8	faint yellow oily solution	Turbid

Table (4): The physical– chemical changes of clonazepam oral drop 2.5 mg/mlwithin time at different temperatures(4).

Storage time (month)	Temp. °C	%of Clonazepam	pH (4-5.5)	appearance
Zero time	R.T	99.5	4.95	Clear faint yellow
1	RT	99.45	4.85	= = =
	40	98.6	4.95	= = =
	50	98.21	4.95	= = =
	60	98.15	4.95	= = =
2	RT	99.40	4.9	= = =
	40	98.53	4.9	= = =
	50	98.21	4.9	= = =
	60	98.1	4.9	= = =
3	RT	99.40	4.80	= = =
	40	98.3	4.85	= = =
	50	98.09	4.85	= = =
	60	97.9	4.85	= = =
4	RT	99.35	4.8	= = =
	40	98	4.8	= = =
	50	98.01	4.8	= = =
	60	97.8	4.8	= = =
5	RT	99.25	4.8	= = =
	40	97.8	4.75	= = =
	50	97.16	4.75	= = =
	60	96.6	4.7	= = =
6	RT	99.2	4.7	= = =
	40	97.65	4.63	= = =
	50	96.21	4.65	= = =
	60	96.1	4.6	= = =

Table (5): The stability of clonazepam oral drop, concentration of clonazepam at zero time =99.48% of the label amount.

Time /day	Concentration at R.T (%)	Concentration at 40° C (%)	Concentration at 50° C (%)	Concentration at 60° C (%)
30	99.45	99.45	98.21	98.15
60	99.40	98.53	98.21	98.1
90	99.40	98.3	98.09	97.9
120	99.35	98	98.01	97.8
150	99.25	97.8	97.16	96.6
180	99.2	97.65	96.21	96.1

Zero – order rate of reaction is expected for kinetic of Clonazepam oral drop particularly in the first stage of reduction of concentration.
T95%=0.05C/K

Where:
C=concentration at zero time
K= rate of reaction
T= time

Table (6): The 95% at different temperature.

Temp. °C	T95% day	T95% Year
25	1095	3
40	1022	2.8
50	912.5	2.5
60	839.5	2.3

Conclusion:

The present study concludes that the formulation developed shows its physiochemical properties like, assay, pH, appearance , and clarity are good.

All the results were comparable with the marketed sample and the drug content also within BP limit.
the dosage forms were stable for a period of(6) months..

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