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RESEARCH ARTICLE

QUALITY EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF COMMERCIALLY AVAILABLEISOTRETINOIN SOFT GELATIN CAPSULES IN SAUDI ARABIA

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ABSTRACT

Isotretinoin (13-cis-Retinoic acid (13-cis-RA)) (INN), it is asynthetic retinoid. In 1982, Isotretinoin was approved as a treatment for severe recalcitrant nodular acne,. There are several generics of isotretinoin soft gelatin capsules available in the pharmaceutical market globally. Availability of various brands of isotretinoin capsules in Saudi drug market today places dermatology physicians in a dilemma. The objective of this study is to compare among the physicochemical properties of four different commercial isotretinoin brands using in vitro tests. The physicochemical equivalence of selected four brands of INN capsules (Roaccutane®, Cureacne®, Xeractan® from local Saudi market and Netlook® from Egypian market), were assessed through the evaluation some tests such as weight variation, weight uniformity (drug content), rupture, disintegration and dissolution for the selected four different brands of soft gelatin capsules. All brands passed all the official tests as prescribed by the USP, all brands were within the limit when tested for weight variation and disintegration time. The drug content of all tested brands was ranged from 90-110%. The rupture test was within the UPS specified values. Additionally, dissolution profile of all tested brands showed a drug release of more than 35% in in the first one hour. This comparative in vitro evaluation study of the teste brands of INN indicate the usefulness, effectiveness and idealness of commercial product. Some variations can be observed as a result of differences in additives used in the capsule formulation manufacturing processes, which may vary from manufacturer to the other. Accordingly, patients can safely switch from one brand to another.

Key words: Accordingly, Patients Can Safely Switch from one Brand to Another.

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INTRODUCTION

The development of a pharmaceutical product is a complex task because the optimal formulation involves many raw excipients and process variables and in the meanwhile. It must meet at least one requirement (responses) such as disintegration, dissolution rates at specific times. In past decades, this task has been achieved through trial and error requiring great expertise and experience which are time-consuming and expensive. Furthermore, it is difficult to determine when and whether the optimal process or composition of excipients has been actually obtained (Tsai *et al*., 2013). Quality control (QC) is the part of Good Manufacturing Practice which refers to the goodness or excellence of a product(Rust and Oliver, 1993). QC emphasizes testing of products for faults and reporting to regulation that makes the decision to investigate or reject the release (Uddin *et al*., 2015). The total quality of the product is assured by both the in process quality control (IPQC) and finished product quality control (FPQC) tests.

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The total dealing process (IPQC and FPQC tests) represents rigorous QC tests to make products completely indefectible before they are delivered into the market(Uddin *et al*., 2015, Uddin *et al*., 2016).

Soft Capsule Dosage Form: Capsule is the most versatile of all dosage forms. Capsules are solid dosage forms in which one or more medicinal and inert ingredients are enclosed in a small shell or container usually made of gelatin(Bhatt and Agrawal, 2008).There are two types of capsules, "hard" and "soft". The hard capsule is also called "two piece" as it consists of two pieces in the form of small cylinders closed at one end, the shorter piece is called the "cap" which fits over the open end of the longer piece, called the "body"(Augsburger, 2002). The soft gelatin capsule is also called as "one piece". Capsules are available in many sizes to provide dosing flexibility. Unpleasant drug tastes and odors can be masked by the tasteless gelatin shell. The administration of liquid and solid drugs enclosed in hard gelatin capsules is one of the most frequently utilized dosage forms(Serajuddin *et al*., 1986, Schiele *et al*., 2013, Brox, 1988, Cimiluca, 1997, Gullapalli, 2010).

Advantages of Capsules

- Capsules mask the taste and odor of unpleasant drugs and can be easily administered.
- They are attractive in appearance
- They are slippery when moist and, hence, easy to swallow with a draught of water.
- As compared to tablets less adjuncts are required.
- The shells are physiologically inert and easily and quickly digested in the gastrointestinal tract.
- They are economical
- They are easy to handle and carry.
- The shells can be opacified (with titanium dioxide) or colored, to give protection from light.

Disadvantages of Capsules

- The drugs which are hygroscopic absorb water from the capsule shell making it brittle and hence are not suitable for filling into capsules.
- The concentrated solutions which require previous dilution are unsuitable for capsules because if administered as such lead to irritation of stomach.

Isotretinoin: The retinoids are a class of compounds which includes both naturally occurring substances with vitamin A activity and synthetic analogues used in dermatology and oncology. Isotretinoin (INN) 13-cis-retinoic acid (13-cis-RA, isotretinoin, Figure 1) is used successfully in the treatment of severe cystic acne and related disorders (G.L. Peck *et al*., 1994). While INN was first developed to be used as a chemotherapy medication for the treatment of some cancers because of its ability to kill rapidly dividing cells(Peck, 1987), it is also considered as one of the most effective drug approved in 1982 to treat, by oral route, severe recalcitrant nodulocystic acne, in spite of its potentially severe side effects, particularly its teragenocity (Guimarães *et al*., 2010). Several generic formulations for oral use have recently been introduced, in addition to the brand formulations Roaccutane® and Accutane ®TM (Roche).

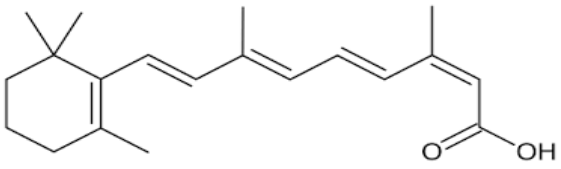

Figure 1. Chemical structure of Isotretinoin

It is used in the treatment of skin disease including acne vulgaris as a topicalkeratolytic agent(Lehucher-Ceyrac and Weber-Buisset, 1993). The mechanism of action is believed to inhibit the secretion of sebum and alter the lipid composition of the skin surface. Its effect on regulating cell differentiation led to use ofit to treat cystic and nodular acne and also to inhibit neoplastic cells proliferation during past decades.INN decreases the sebum production and shrinks clearly the sebaceous glands. Isotretinoin prevents comedones from forming and stabilizes keratinization. It decreases inflammation in severe inflammatory acne. (Nelson *et al*., 2009, Akyol and Özçelik, 2005). The complete therapy course consists of three to four months of isotretinoin intake(Amichai *et al*., 2006).

Due to its elevated lipophilicity, oral absorption of INN is improved when given with a fatty meal. After oral intake of isotretinoin on empty stomach and with fatty meals, both C_{max} and the AUC of isotretinoin were more than doubled subsequent to a standardized high fatty meal when compared with isotretinoin given under fasted conditions. Females must avoid pregnancy while taking isotretinoin or for at least 30 days after the drug discontinuation that is because isotretinoin results in severe birth defects in a high percentage of infants who born to female who became pregnant during isotretinoin intake in any amount, isotretinoin is a dangerous teratogen (Hill, 1984). INN is also contraindicated in the following cases: breast feeding female, renal and hepatic insufficiency, hypervitaminotic A, patients with elevated blood lipid levels, patients who take tetracyclines and isotretinoin sensitive patients. Isotretinoin intake has been associated with cases of benign intracranial hypertension(Goodfield *et al*., 2010). INN should be. preserve in tight containers, protected from light. Store at controlled room temprature, in a dry place(Cafiero, 2017, Revision, 1984, Micronized, 2012).

Objective of the Present Study: Quality Control or in vitro testing of drug products is a set of studies undertaken during in process and post production by regulatory agencies and researchers. Routine laboratory testing of the drugs commercially available in the market is a crucial to ensure that the final products follow the official international standard and thus protect public health. Therefore, it was important and interested to emulatethree different brands of INN soft gelatin capsule, which are commercially available in local markets of Saudi Arabia, named as follow Cureacne®, Xeractan® with addition of Netlook® which bought from Egypt in comparison to pioneer generic formulation Roaccutane®, all are formulated as soft gelatin capsules. The evaluation was performed according to United States Pharmacopeia (USP) through in vitro evaluation of several parameters including content uniformity, weight variation, disintegration time, rupture and dissolution tests. Other characterizingtests performed in the study include organoleptic physical characterization of the soft gelatin capsule and determination of thickness and diameter, to ensure the pharmaceutical quality of the investigated brands.

MATERIALS AND METHODS

Materials: Pure isotretinoin authentic powder from Aljazeera pharmaceutical company, Riyadh, Saudi Arabia. sodium hydroxide pellet (BDH, GBR, England).hydrochloric acid 37% (BDH, GBR, England). All reagent used were of analytical grade. Freshly distilled water was used throughout the work and Ethanol. Isotretinoin, having label strength of 20 mg of four different brands were purchased from trusted registered pharmacies in Riyadh, Saudi Arabia and Cairo, Egypt as illustrated in Table 1.

Instrumentation: List of instruments utilized in the present study is illustrated in the following table:

Methods

Calibration Curve

Preparation of standard solution of INN and Construction of calibration curve: The standard stock solution was prepared by dissolving 50 mg of INN slandered solution in methanol in 10ml amber colored volumetric flask and volume was made up to the mark (5 µg/ml).

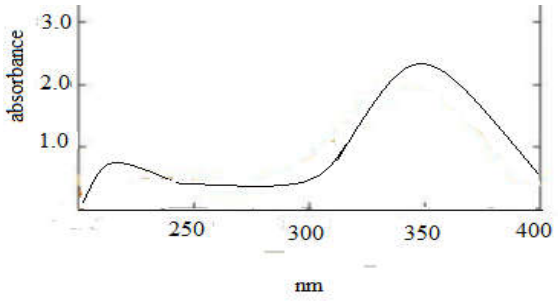

Figure 2. The Spectrum of Isotretinoin in methanol

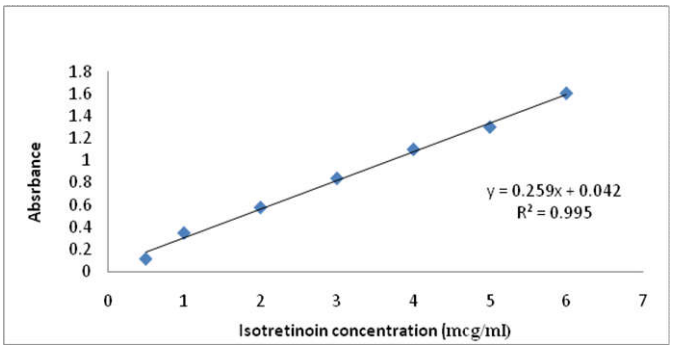

Figure 3. Calibration Curve of Isotretinoin reference standard at λ344 in methanol

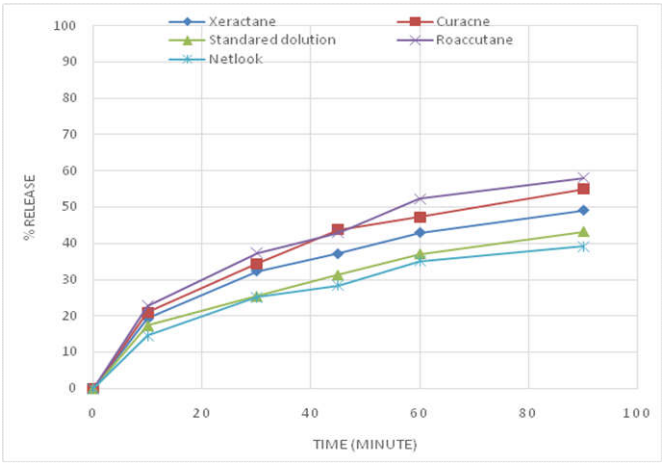

Figure 4. *In vitro* drug release behavior for INN from

The stock solution was suitably diluted with methanol to obtain sample having concentrations of 3 µcg /ml and this was then scanned in UV range from 200-400 using UV spectrophotometer. The maximum wavelength was found to be 344 nm, as shown in Figure 2. Appropriate serial dilutions of the stock solution 5mg/ml were prepared to obtained different concentrations of INNas 0.5, 1, 2, 3, 4, 5 and 6 µcg /ml, the volume was completed up by methanol. The absorbance measurements of these solutions were carried out against methanol as blank and measured spectro photometrically at λ maximum of 344 nm. Triplicate absorbance values of each concentration were measured. The results were plotted against the corresponding standard concentrations to obtain the calibration curve and the corresponding regression equation was delivered.

Unknown concentration of INN was determined by using the equation (1) of standard curve.

$$Y = Mx + b \quad (1)$$

Where: Y=absorbance; X=concentration µg /ml, M=slope and b=intercept.

Effect of storage Condition

Effect of storage of standard solution at refrigerator (4^0 C) protect from light: The stability of INN as different concentrations 0.5,1,2,3,4,5 and 6 µcg/ml in refrigerator and protect from light were monitored for one week. The absorbance of these solutions were measured by UV spectrophotometric. Each sample was assessed at the beginning of the study, after seven days of storage, triplicate reading was recorder.

Effect of storage of standard solution stored at room temperature protect from light: The stability of INN as different concentrations 0.5,1,2,3,4,5 and 6 µcg/ml stored under ambient conditions (at room temperature, 25 ^{0}C ± 1) and protect from light were monitored for one week. The absorbance of these solutions were measured by UV spectrophotometric. Each sample was assessed at the beginning of the study, after seven days of storage, triplicate reading was calculated.

Effect of storage of standard solution stored at room temperature in direct sun light: The photochemical behavior of INN was investigated, the different concentrations of INN 0.5,1,2,3,4,5 and 6 µcg subjected to direct sunlight for oneweek .the reaction was followed by measuring the absorbance by UV spectrophotometer and the percentage of loss then calculated, triplicate reading was calculated.

Effect of storage of standard solution stored at oven and protect from light: Effect of storage INN standard solution in dark at 450 C and 75% humidity (in oven) was investigated, the different concentrations of INN 0.5,1,2,3,4,5 and 6 µcg subjected are kept in oven for one week. the reaction was followed by measuring the absorbance by UV spectrophotometer and the percentage of loss then calculated. Triplicated experiments were done for each tested concentrations.

Quality Control Tests of Isotretinoin soft Gelatin capsule

Soft Gelatin capsule Physical properties: Outer shape, color, thickness and diameter of the four test brand were examined visually. The thickness was measured individually for ten soft gelatin capsules from each brand by utilizing the Micrometer. The average of ten determination weight and standarddeviation (± SD) were calculated.

Uniformity of Weight (Weight variations): A batch of twenty (20) capsules of each brand of the fours tested brands were selected randomly. Each capsule was weight individually on electronic analytical balance then the average weigh (W) and (SD) were calculated. The percentage (%) deviation was determined by comparing each individually capsule weight against the calculated average using equation (2)

$$\% \text{ difference} = \frac{\boldsymbol{indivdual\ capsule\ weight(gm) - W(gm)}}{\boldsymbol{W(gm)}} \times 100 \quad (2)$$

Content Uniformity: Isotretinoin content was evaluated according USP requirements for content uniformity (USP 34/NF 29,2011). A batch of twenty (20) capsules of each brand

of the fours tested brands were selected randomly. Each soft gelatin capsule was weight individually on electronic analytical balance the average weigh (AW) and (SD) were calculated. After that cut and opened each capsule, remove the INN content by washing three times wash suitable solvent (chloroform: methanol (3:1). After that allow the solvent to evaporate from the shell at room temperature. After drying weight, the individual shell, calculate the net content by using equations (3) and (4)

Net content of isotretinoin =weight of capsule (gm) -weight of empty shell (gm) (3)

$$\% \text{ of content of isotretinoin} = \frac{\text{Net content (gm)}}{\text{avarage of net content}} \times 100 \quad (4)$$

Rupture test: Rupture test involved use of dissolution apparatus2, water as a medium for capsules and the paddles operating at 50 rpm. The rupture test was performed in 500 mL of water at 37 °C and 50 rpm using USP dissolution apparatus 2. Six capsules were selected randomly. The test capsules are dropped in the dissolution vessel containing 500 mL of water. Soft gelatin capsules are well observed and the time taken for the capsule outer shell to rupture is noticed. Capsules pass the test if six of them rupture in not more than 15 minutes, if one or two capsules rupture more than 15 minutes; the test is repeated with another additional 12 capsules. Out of these 18 capsules, just only two are allowed to rupture more than 15 minutes but should rupture within 30 minutes. Similar test was done by Jasmin (Jasmin Lalji, 2012*)*.

Disintegration test: Disintegration test for the four brands was performed according to USP procedure using a disintegration apparatus. The USP disintegration apparatus consist of six glass tubes that are 3 inches long, open at the one capsule is placed in each tube and the basket rack is positioned in specified medium (water) at 37±2ºC such that capsule remains 2.5 cm below the surface of the liquid on their upward movement and descend not closer than 2.5 cm from the bottom of the beaker. A standard motor driven device is used to move the basket assembly containing the capsules up and down through distance of 5 to 6 cm at a frequency of 28 to 32 cycles per minute. Similar test was done by Jasmin (Jasmin Lalji, 2012).Table 3 showed the differences between disintegration test (USP chapter 701) and rupture (USP chapter 2040).

Determination of in vitro Dissolution rate of isotretinoin: Caution- carry out all the test protect from light. Dissolution test of INN from the four brands was performed according to USP procedure using a disintegration (Disintegration-701)— No disks; the apparatus is adjusted so that the bottom of the basket-rack assembly descends to 0.1±1.0 cm from the inside bottom surface of the vessel on the downward stroke; the 10-mesh stainless steel cloth in the basket-rack assembly is replaced with a 40-mesh stainless steel cloth; a 10- mesh stainless-steel cloth is fitted to the top of the basket-rack assembly. The dissolution medium was consist of 100 ml of stage 1 (simulated gastric fluid with pepsin, prepared freshly and purged with nitrogen), and + 800 ml of stage 2 (0.13 N sodium hydroxide) at 37.0 ± 0.5 ˚C Standard solution— Transfer about 10 mg of USP Isotretinoin RS, accurately weighed, to a 200-mL low-actinic volumetric flask; add 25.0 mL of Stage 1 Medium and about 150 mL of Stage 2 Medium; sonicate until completely dissolved (about 20 minutes); and dilute with Stage 2 Medium to volume. Pass 20 mL of this solution through a suitable filter, discarding the first 5 ml. Dilute 5.0 mL of filtrate with Stage 2 Medium to 50 ml. Read UV spectrophotometrically obtain a theoretical concentration of about 0.0055 mg per ml of isotretinoin, assuming complete dissolution, based on the label claim. Sample solution— Perform a dissolution test on each of 6 Capsules: place 1 Capsule in one of the tubes in each of six basket-rack assemblies. With the disintegration apparatus operating, connect each basket-rack assembly to the drive rod in a timed sequence. After 10, 30, 45, 60 and 90 minutes, withdraw 20 mL of Medium (Stage 1 and Stage 2), immediately pass the solution through a suitable 0.45-mm filter, discard the first 5 ml. take 5 ml of the filtrate compete to 50 ml by stage 2 solution. The absorbance was measured using UV-visible spectrophotometer at λmax of 344 nm. The concentration of INN released from the capsule at each interval was calculated from the calibration curve of INN. The amount and the percentage of INN released from the capsule at each time interval was determined from according to equation (5) and (6) respectively.

Amount of drug dissolved (mg)

$$= \frac{\text{concentation} \times \text{dissolution bath volum} \times \text{dilution factor}}{1000} \quad (5)$$

Percentage of drug dissolved

$$= \frac{amount\ of\ drug\ dissolved\ (mg)}{20(drug\ content\ of\ capsule)} \times 100 \quad (6)$$

RESULTS AND DISCUSSION

Calibration curve of INN in methanol: An absorption maximum of INN in methanol was found to be at 344nm.A calibration curve of peak area ratios of INN versus concentrations ranging from 0.5 to 6.0 mcg/ml, was plotted in Figure 3. Each point on the calibration curve represents a mean of six determinations. A linear curve was obtained for the range of concentration tested with a correlation coefficient (*r*) of 0.9953. The regression equation was Y = 0.2594X + 0.0422. Therefore, good relationships between the INN peak area ratios and its tested concentrations. Table 1 and Table 5, showed the interday and intraday precession (abs) (n=9).

Effect of the storage condition on the standard solution of INN in methanol: The photostability of INN represents a significant problemin pharmaceutical research, several articles and reviewsdealing with different aspects of such studies(De Villiers *et al*., 1992). Standard solution preparation of INN in the concentration range from 0.5- 6mcg/ml in methanol were stored in different conditions, i.e. at refrigerator, at room temperature and at high temperature to investigate the photostability of INN. After one week measurement of three triplicate of each concentration s were read as UV spectrophotometrically. The results shown in Table 6 which indicate that INN is very stable if prepared and sobered either in refrigerator (at 4^0C) or in room temperature (25^0C) protect from light for one week, less than 1 and 8% degradation respectively. Additionally, INN standard solution with same concertation's show massive degradation more than 40% loss in concentrations if stored either at direct sun light or at high temperature (45^oC). As a result INN should be protecting from direct sunlight and from high temperature.

Table 1. List of four tested brands of Isotretinoin soft gelatin capsule

Brand	Manufacture	Batch No.	Manufacturing date	Expiry date
Roaccutane®	Hoffman-La Roche, Basel. Switzerland	B9281B02	09/2015	09/2018
Cureacne®	Pierre Fabre Pharmaceutical, France	HR066466A	08/2015	08/2018
Xeractan®	Pharmathen pharmaceutical for Aljazeera pharmaceutical industries, Riyadh, Saudi Arabia	1605722	09/2016	09/2018
Netlook®	Al Andalous Medical Company, Cairo, Egypt	160595	12/2016	11/2019

Table 2. List of apparatus used in experiments

Instruments name	Manufacturer
UV visible spectrophotometer	Libra S22(biochrom, England)
Sonicator	Elmasonic X-tra 150 H, Elma, Singen, Germany)
Electronic Balance	JBI603-c/FACT, Mettler - Toledo, Switzerland)
Disintegration test apparatus	Pjarma Test PTZS-Handburg, Germany
Dissolution Test apparatus	Pharma Test PTZSS(Handburg, Germany
Micrometer	Vernier caliper, Germany

Table 3. Differences between disintegration test (USP chapter 701) and rupture (USP chapter 2040)

Difference	Disintegration test	Rupture test
Apparatus	*Six cell basket rack assembly*	*Dissolution apparatus 2*
Volume of test medium	*1000 mL*	*500 mL*
Hydrodynamics	*Up/down strokes*	*Rotary paddle*
Duration	*NMT 45 minutes*	*15 minutes*
End point	*No palpable firm core*	*Rupture of capsule shell*

Table 4. Interday precession same day (abs)(n=9)

Concentration (µg/ml)	0.5	3	6
Average	0.08	0.83	1.63
SD	0.026	0.008	0.03
RSD%	30.32	0.95	1.62

Table 5. Interaday precession on three different days (abs)(n=9)

Concentration (µg/ml)	0.5	3	6
Average	0.49	3.04	5.91
SD	0.008	0.04	0.28
RSD%	1.63	1.51	4.74

Table 6. Stability of Isotretinoin in different storage conditions

Concentration (µg/ml)	Mean %loss ±SD			
	after one week at refrigerator (4^0C) protect from light	after one week at room temperature protect from light	after one week at room temperature under direct sun light	Absorption after one week at 45 ^{0}C and 75% humidity
0.5	0.13 ± 1.24	0.674 ± 2.45	49.41 ± 9.81	45.54 ± 14.85
1	0.11 ± 1.31	0.144 ± 3.10	47.06 ± 22.04	9.89 ± 23.05
2	0.15 ± 1.11	7.745 ± 1.91	51.76 ± 17.21	18.72 ± 1.81
3	0.23 ± 1.07	7.13 ± 1.71	49.82 ± 13.87	20.09 ± 1.13
4	0.26 ± 1.13	6.33 ± 1.39	56.67 ± 2.77	8.87 ± 1.56
5	0.45 ± 1.09	4.52 ± 1.79	60.20 ± 5.59	22.25 ± 0.05
6	0.51 ± 1.02	1.912 ± 1.22	66.05 ± 2.01	18.04 ± 1.62

Table 7. Organoleptic properties of the tested four tested brands (n=10)

Brand	Shape	Color	Thickness (mm) (average ±SD, n=10)	Diameter(mm) (average ±SD, n=10)
Roaccutane®	Oval	Pink and white	13.858 ±0.07	8.04 ± 0.08
Cureacne®	Oval	Red and off-white	12.84 ± 0.05	8.04 ± 0.06
Xeractan®	Oval	brawn red	12.83 ± 0.02	7.75 ± 0.04
Netlook®	Oval	Pink and white	12.76 ± 0.04	7.77 ± 0.05

Similar observation was seen by (Ioele *et al*., 2005;Brisaert *et al*., 1995; Tashtoush *et al*., 2008; Boehlert, 1984)

Capsule Physical properties: Capsule physical prosperities are very important for the patient computability. Capsule thickness, shape and color must be evaluated appropriately as those prosperities are essential for consumer acceptance of the product and to facilitate packaging. All those physical properties of the four tested brands are within the accepted limits.

There is a little difference in thickness and diameter of the capsules as shown in table 7 that attributed the excipients that added in different amount according to each company. The differences between brands in the thickness probably depend on the added excipients in different amounts according to each company's formulation. The results revealed that all the commercially tested capsules showed acceptable appearance, organoleptic properties, thickness and diameter.

Table 8. Weight variation and drug content of Roaccute ® 20 mg capsule (n=20)

Capsule	Total weight (gm)	% Difference	Shell weight (gm)	Net content (gm)	Content uniformity(%)
1	0.514	0.300	0.189	0.325	100.277
2	0.523	-1.445	0.179	0.344	106.140
3	0.513	0.494	0.221	0.292	90.095
4	0.509	1.270	0.185	0.324	99.969
5	0.512	0.688	0.195	0.317	97.809
6	0.506	1.852	0.167	0.339	104.594
7	0.509	1.270	0.199	0.31	95.649
8	0.51	1.076	0.187	0.323	99.660
9	0.509	1.270	0.187	0.322	99.352
10	0.524	-1.639	0.208	0.316	97.500
11	0.525	-1.832	0.209	0.316	97.500
12	0.511	0.882	0.169	0.342	105.522
13	0.531	-2.996	0.171	0.36	111.076
14	0.524	-1.639	0.22	0.304	93.798
15	0.502	2.628	0.183	0.319	98.426
16	0.516	-0.087	0.197	0.319	98.426
17	0.513	0.494	0.196	0.317	97.809
18	0.522	-1.251	0.185	0.337	103.980
19	0.523	-1.445	0.201	0.322	99.352
20	0.515	0.106	0.181	0.334	103.054
Average	0.516		0.191	0.324	100.000
SD	0.008		0.015	0.015	4.706
CV%	1.481		8.009	4.706	4.706

Table 9.Weight variationand drug content of Curacne ® 20 mg capsule (n=20)

Capsule	Total weight (gm)	% Difference	Shell weight (gm)	Net content (gm)	Content niformity (%)
1	0.489	0.296	0.160	0.329	101.684
2	0.491	-0.112	0.160	0.331	102.302
3	0.494	-0.724	0.188	0.306	94.575
4	0.495	-0.928	0.190	0.305	94.266
5	0.497	-1.336	0.156	0.341	105.393
6	0.486	0.907	0.162	0.324	100.139
7	0.491	-0.112	0.180	0.311	96.121
8	0.492	-0.316	0.126	0.366	113.120
9	0.491	-0.112	0.183	0.308	95.193
10	0.495	-0.928	0.181	0.314	97.048
11	0.492	-0.316	0.183	0.309	95.503
12	0.491	-0.112	0.161	0.330	101.993
13	0.490	0.092	0.151	0.339	104.775
14	0.490	0.092	0.193	0.297	91.794
15	0.481	1.927	0.144	0.337	104.157
16	0.492	-0.316	0.131	0.361	111.574
17	0.491	-0.112	0.206	0.285	88.085
18	0.475	3.150	0.135	0.340	105.084
19	0.493	-0.520	0.160	0.333	102.920
20	0.493	-0.520	0.188	0.305	94.266
Average	0.490		0.167	0.324	100.00
SD	0.005		0.022	0.021	6.462
CV%	1.013		13.468	6.462	6.462

Table 10. Weight variation and drug content of Xerctan ® 20 mg capsule (n=20)

Capsule	total weight (gm)	% difference	shell weight (gm)	net content (gm)	Content uniformity(%)
1	0.504	0.454276121	0.189	0.315	100.0476417
2	0.509	-0.53328066	0.179	0.33	104.8118152
3	0.508	-0.33576931	0.221	0.287	91.15451802
4	0.509	-0.53328066	0.185	0.324	102.9061458
5	0.508	-0.33576931	0.195	0.313	99.41241861
6	0.503	0.651787478	0.167	0.336	106.7174845
7	0.508	-0.33576931	0.199	0.309	98.14197237
8	0.509	-0.53328066	0.187	0.322	102.2709227
9	0.508	-0.33576931	0.187	0.321	101.9533111
10	0.508	-0.33576931	0.208	0.3	95.28346832
11	0.506	0.059253407	0.209	0.297	94.33063364
12	0.511	-0.92830338	0.169	0.342	108.6231539
13	0.507	-0.13825795	0.171	0.336	106.7174845
14	0.511	-0.92830338	0.22	0.291	92.42496427
15	0.502	0.849298835	0.183	0.319	101.318088
16	0.502	0.849298835	0.197	0.305	96.87152612
17	0.507	-0.13825795	0.196	0.311	98.77719549
18	0.501	1.046810192	0.185	0.316	100.3652533
19	0.503	0.651787478	0.201	0.302	95.91869144
20	0.502	0.849298835	0.181	0.321	101.9533111
Average	0.506		0.191	0.315	100.000
SD	0.003		0.015	0.015	4.772
CV%	0.628		8.009	4.772	4.772

Table 11. Weight variation and drug content of Netlook®20 mg capsule (n=20)

Capsule	Total weight (gm)	% difference	shell weight (gm)	net content (gm)	Content uniformity(%)
1	0.524	0.2379	0.182	0.342	100.249
2	0.526	-0.142	0.198	0.328	96.145
3	0.514	2.141	0.187	0.327	95.852
4	0.538	-2.427	0.201	0.337	98.783
5	0.529	-0.713	0.215	0.314	92.041
6	0.531	-1.094	0.218	0.313	91.748
7	0.529	-0.713	0.155	0.374	109.629
8	0.512	2.522	0.198	0.314	92.041
9	0.531	-1.094	0.178	0.353	103.473
10	0.533	-1.475	0.152	0.381	111.681
11	0.491	6.520	0.185	0.306	89.696
12	0.549	-4.521	0.193	0.356	104.352
13	0.546	-3.950	0.176	0.37	108.456
14	0.522	0.618	0.174	0.348	102.007
15	0.528	-0.523	0.145	0.383	112.267
16	0.53	-0.904	0.199	0.331	97.024
17	0.544	-3.569	0.159	0.385	112.853
18	0.489	6.901	0.182	0.307	89.989
19	0.531	-1.094	0.198	0.333	97.611
20	0.508	3.284	0.187	0.321	94.093
Average	0.525		0.184	0.341	100.000
SD	0.016		0.020	0.026	7.731
CV%	3.034		10.819	7.731	7.731

Table 12. Rupture time in minutes for tested ISO brands capsule (n=6)

Brand	1	2	3	4	5	6	Rupture time (min) (Average ± SD)
Roaccutane®	03:24	03:44	03:50	03:30	03:45	3:50	3.39 ± 0.01
Cureacne®	02:02	02:10	02:15	02:15	02:13	02:12	2.10 ± 0.01
Xeractan®	05:00	06:40	04:11	06:20	05:55	6:00	5.38 ± 0.04
Netlook®	05:29	06:30	09:06	09:17	08:36	09:03	7.53 ± 0.07

Table 13. Disintegration time test in minutes for tested INN brands capsule (n=6)

Brands	Average ± SD
Roaccutane®	14.12 ± 0.53
Cureacne®	12.80 ± 0.90
Xeractan®	13.05 ± 0.06
Netlook®	13.97 ± 0.77

Weight variation and Content Uniformity: The weight of the capsule should be evaluated to ensure that the capsule contains the proper amount of the active ingredient(Gad, 2008). The weight variation of the four tested brands of INN was determined and the results are shown in Table 8-11. The USP provides guidelines for capsule weight variations, weight was expressed as percentage of the average weight of the sample. The percentage differences of the test of the weight of the individual capsules falls within the average range (less than 2% variation). If the requirement is not met, the weight of the content s for each individual capsule is determined and compared with the average weight of the content. The uniformity of weight for all the four brands gave values complied with official standard book specifications for weight uniformity. None of the capsules has deviated by up to ± 10% from the mean standard value as shown in tables 8, 9, 10 and 11. Content uniformity test has been performed for the four brands, although there is a deviation in few capsules but generally the entire four brands met the required standard specification as shown in Table 8-11. Isotretinoin Soft gelatin capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of isotretinoin (USP 36/NF31 ,2012). On analysis the average INN content of four products, all tested samples fell inside the USP specifications of 90-110%, none of soft gelatin capsule fall outside the range none of the tested soft gelatin capsule fall outside the specified range.

Rupture test: The time required for capsule rupture has been determined for the four brands. All of them were within the accepted range; none of them has exceeded 15 minutes for rupture as illustrated in table 12.

Disintegration test: The disintegration test has been performed for the four brands capsule. All of them met the required speciation as shown in table 13.None of the tested soft gelatin capsule fall out side the specified range

In vitro Release of Isotretinoin: In vitro drug release profiles an imperative test to predict the in vivo performance of a drug delivery system. An in vitro drug release study is indeed a prerequisite for a drug design optimal effect (Kumari *et al*., 2010). The drug release pattern was studied for 90 minutes for all tested brands in comparison to slandered solution. The *in vitro* release profiles of INN in disintegration apparatus containing 900 ml of dissolution medium, 100 ml of stage 1 and 800 ml of stage 2 were depicted in Figure 4. The differences observed in release profiles may be due to differences in the additives in the formulations. The percentage drug release was analyzed as shown in (Figure 4). The average percentage release from all brands after 60 min (Table 14) was rang 0.006 to0.007 The release profile of tested capsules met with the criteria of the USP a theoretical concentration of about of releasing not less than 0.0055 mg per ml.

Conclusion

The results have been shown that all the teste brands of INN satisfied the USP requirements in terms dissolution. Dissolution profiles released that all the tested INN brands were equivalent to the originator (Roaccutane). Accordingly, patients can safely switch from one brand to another. Some variations can be observed as a result of differences in additives used in the capsule formulation manufacturing processes, which may vary from manufacturer to the other. As general all their physicochemical properties were quite comparable to each other for all tested brands.

Conflict of interest statement: We declare that we have no conflict of interest.

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