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DIETARY SUPPLEMENTS – BIOACTIVE PRINCIPLES: REGULATORY ASPECTS, *IN VITRO* RELEASE PROFILES – COENZYME Q10

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ABSTRACT: Nowadays, dietary supplements represent an important part of the products that people use in order to support daily health. Figures show a steady increase in the consumption of dietary supplements at people of all ages, in many cases associated with chronic medicinal treatment. In this review we have presented an insight into dietary supplements regulations in Europe, Romania and United Stated of America, quality control tests and methods (compendial and non compendial) used to determine the characteristics of some pharmaceutical oral forms (capsules, soft gelatin capsules, tablets), and performance test, *in vitro* release profiles study. Also, we presented an overview on Coenzyme Q10, current status and uses, as well as different formulation attempts to improve the release profile data of this bio-active principle.

Keywords: regulatory, dietary supplements, bio-active, release profiles, coenzime Q10

INTRODUCTION:

The number of dietary supplements in Romania is continually growing, economic data show that the market has risen in this respect to 500 million euros in 2015, according to Cătălin Vicol, the president of the Romanian Employers of Dietary Supplements Industry.

Following the diversity of pharmaceutical forms available in pharmacies, drugstores, online media, the need for quality assurance is becoming greater. Regulatory aspects as well as good manufacturing practice guidelines establish clear regulation regarding quality norms that should be met by every product sold for human health support.

From the early stages of drug development, dissolution test is one of the predicting factors of the kinetic performance of the future product. It is a holistic test that can indicate the release profile of the active pharmaceutical ingredient in time and an insight into the bio-availability of the product. (Scheubel E.,2010)

Biopharmaceutical Classification System (Amidon et al, 1995) rates active substances based upon their solubility and permeability:

Class I BCS – highly soluble and permeable active substances, with high bio-availability

Class II BCS – low solubility and high permeability

Class III BCS – high solubility and low permeability

Class IV BCS – low solubility and low permeability

This classification system was developed in order to reduce the need for *in vivo* studies (bio equivalence studies). BCS classification system takes into consideration three acting factors involved in immediate release drugs profiles: dissolution rate in time, solubility and intestinal permeability.

Table 1

IVIVC expectations for IR products based on the BCS Classification (Mohd Yasir et al., 2010)

Class	Permeability/Solubility	Absorption rate control step	IVIVC
Class I	High/High	Gastric emptying	IVIVC expected if dissolution rate is slower than gastric emptying rate. Otherwise limited or no correlation
Class II	High/Low	Dissolution	IVIVC expected if *in vitro* dissolution rate is similar to *in vivo* dissolution rate, unless dose is very high
Class III	Low/High	Permeability	Absorption is rate determining and Limited or no IVIVC with dissolution
Class IV	Low/Low	Case by case	Limited or no IVIVC expected

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A medicinal product is considered highly soluble if it dissolves in an aqueous volume of 250 ml, pH varying from 1.0 to 7.5. Permeability of an active substance is determined either directly by measuring the rate of intestinal absorption (blood determination), or indirectly by measuring percent mass transfer using a membrane. An active pharmaceutical ingredient is considered highly permeable if 90% of the tested mass passes the intestinal membrane; also, it is considered rapidly dissolving if 85% of the mass is dissolved using Apparatus USP I with a rotation rate of 100 rpm, or Apparatul USP II with a rotation rate of 50 rpm in a volume of 900 ml (or 500 ml) dissolution media (acid 0.1 N HCl or gastric simulation fluid without enzymes or, at pH 4.5 buffer, or pH 6.8 alkaline buffer).

There are different methods that can be used in order to evaluate the *in vitro* release profile of products. Among these we mention devices that function in non-sink conditions - natural convection (static disc, suspended paddle) or forced convection (magnetic basket, rotating disk method, paddle, rotating basket apparatus). There are also devices with one, two or three compartments separated by a semipermeable membrane.

Dissolution test is one of the most used kinetic method for the evaluation of a pharmaceutical product's release profile and quality analysis. Based upon the specifications of the product, batch release is performed to the market.

United States Pharmacopoeia describes quality control tests in the following chapters: <701> Disintegration, <711> Dissolution, <724> Drug Release, <1092> The Dissolution Procedure, Development and Validation, <1094> Liquid-filled Capsules—Dissolution Testing and Related Quality Attributes. Dissolution Guides contain indications regarding drug release profiles comparison and using the dissolution test -*in vitro* test - as an equivalent to - *in vivo* test-biowaver. (Shah V.,2015)

DISSCUSIONS

Regulatory aspects in European Union and Romania

According to European Comparison Directive 46/2002 "...dietary supplements are nutritional products that have the purpose to complete the normal diet and are concentrated sources of nutrients or other nutritional or physiological substances separately or combined marketed as dosage forms...", Nutrients can be: vitamins, minerals.

European legislation regarding dietary supplements has been transposed in Romanian legislation and numerous Regulations adopted concerning: maximum levels of impurities (e.g. metal), labeling, food additives.

According to a study conducted by the European Advisory Services (EAS) for DG SANCO, EUROPEAN COMMISSION on the 28th of 2007 about THE USE OF SUBSTANCES WITH NUTRITIONAL OR PHYSIOLOGICAL EFFECT OTHER THAN VITAMINS AND MINERALS IN FOOD SUPPLEMENTS these can be divided into 6 categories: amino-acids, enzymes, probiotics, botanical, essential fat acids and other active biological substances like Coenzyme Q10, lycopene, glucosamine.

In Romania, a dietary supplement must be notified to the Ministry of Health in order to obtain the approval for marketing release. (Garban G.,2013)

On the 2nd of November 2016, The European Medicines Agency (EMA) has designated Ubiquinol as an orphan drug for the treatment of primary coenzyme Q10 deficiency, a serious rare disease which produces muscular, nervous system and kidney disorders and can cause failures of other organs.

Regulatory aspects in United States of America

Since the 1940s the USA has started introducing regulations for dietary supplements because of the increasing numbers of these products marketed. In 1958 Food and Drug Administration (FDA) applied the Food Additives Amendment to dietary supplements extracted from plants. The manufacturer of the food additive had the responsibility to prove to the authorities that the product was safe, based on existing information. In 1994 USA adopted DSHEA (The Dietary Supplements and Health Education Act) which brought a significant change, the burden of proving that a dietary supplement was safe was no longer on the shoulders of the manufacturer, but it was FDA that was responsible for proving that a dietary supplement or an ingredient from it was forged. Also, DSHEA took dietary supplements out of the food additives category. *(Complementary Alt.Med.[2005],258).*

Dietary supplements that were commercialized after DSHEA had to be notified to FDA with 75 days before actual marketing. The notification had to contain information about the new supplement, prove that it was safe. If FDA could not prove the opposite, the supplement was free to be marketed.

In November 2001 USP-NF launched „The Dietary Verification Program"(DSVP), with the purpose of increasing dietary supplement's safety. The products that were included in this program were given a certificate that proved that the information on the label was real, the impurities were in permitted limits, the current general monograph from USP-NF were respected.

In 2003 FDA proposed a set of guidelines for the Good manufacturing practice of dietary supplements.(Framework 2005, 55-60).

Nowadays there are three programs of certification for dietary supplements in USA: ConsumerLab.com, NSF International and US Pharmacopeial Convention.*(Akabas SR el al [2016]).*

The general chapter USP-NF<Manufacturing practices for dietary supplements> mentions aspects about quality control, personnel, equipment used for manufacturing, cleaning, testing of materials used, labeling, process controls, distribution.

Compendial Methods for quality control tests

USP-NF (United States Pharmacopeea-National Formulary) mentions general chapters <701> Disintegration and <711>Dissolution as being

mandatory in *in vitro* release profile documentation; the latter chapter mentioned contains the description of compendial Apparatus (4, the other 3 compendial apparatus are described in the general chapter <724>), working procedures, results interpretation and acceptance criteria.

The European Pharmacopoeia contains the general chapter 2.9.3 Dissolution tests for oral sold forms, in which compendial Apparatus (4) are presented as well as working procedures, results interpretation , acceptance criteria and information on qualification and validation methods.

The Japanese Pharmacopoeia contains the general chapter 6.10 Dissolution tests- general procedures and apparatus (3). (Stippler E., 2011)

Table 2

An updated review on Dissolution Apparatus for conventional and novel dosage forms (Sree. C. Lakshmi, 2013)

Sr. No	Official Name	Main features of the apparatus	Uses
1	USP Apparatus 1	Basket	Tablets, capsules, floating dosage forms
2	USP Apparatus 2	Paddle	Tablets, capsules, enteric forms
3	USP Apparatus 3	Reciprocating cylinder	Extended release drug products
4	USP Apparatus 4	Flow through cell	Implants, powders, suspensions
5	USP Apparatus 5	Paddle over disk	TDDS
6	USP Apparatus 6	Cylinder	TDDS
7	USP Apparatus 7	Reciprocating disk	Extended release drug products

USP Apparatus 1 – Basket

The historical USP 40 mesh dissolution basket has 40 openings per linear inch. Openings are equal in both directions producing a standard square weave. USP specifies that 40 mesh (40 x 40) screen be manufactured with wire having a nominal 0.25mm diameter. Harmonized basket specifications are now referred to as "0.22-0.31 mm wire diameter with wire openings of 0.36-0.44 mm.". Products usually tested using Apparatus 1 are: capsules, tablets, floaters, modified release, suppositories (modified Palmeri basket). The basked is placed in a 1000 ml capacity vessel, with dissolution media, rotation speed (50-100 rpm) and temperature predefined. Lately, the dissolution volume is considered to be 500 ml instead of 900ml.. The disadvantages of this apparatus are that formulation may clog the screen and small disintegrated particles fall out of the basket.

USP Apparatus 2- Paddle

USP Apparatus 2, the rotating paddle method, followed the development of the rotating basket method with better stirring characteristics. The paddle blade is fixed to the bottom of the shaft and rotates at a height of 25mm from the inner bottom of the vessel.

The paddle apparatus consists of a metallic or suitably inert, rigid blade and shaft comprising a single entity. The paddle blade and shaft may be coated with a suitable inert material. The paddle is lowered into a 1L vessel and rotated at a specific speed within media which is maintained at a specific temperature. The rotating paddle method is routinely used at an agitation speed of 25 to 75 rpm. Rates outside a range of 25 to 75 rpm are generally unacceptable because of the lack of reproducibility of the hydrodynamics below 25 rpm and turbulence above 100 rpm. High turbulence in the vessel leads to a loss of discriminatory power associated with the method.

A small, loose piece of non-reactive material such as not more that a few turns of wire helix may be attached to dosage units that would otherwise float. In addition to sinking floating dosage forms, sinkers may assist in keeping a dosage form from sticking to the vessel inappropriately as in the case with some film coated tablets. Sinkers must be adequately described in the method to eliminate hydrodynamic variation associated with different sinker devices. Drug products tested using this apparatus are: tablets, capsules, suspensions, powders. As disadvantages of this apparatus we mention: floating dosage forms require sinkers, cone formation may be problematic, and positioning of the dosage form in the vessel may also be difficult. Cone formation is a typical problem for disintegrating products (especially if hydrophobic), fluid is interchanged only at surface, in the center of the cone there may be saturated solution. Increasing rotation may overcome the problem. A PEAK vessel with an inverted cone molded into the bottom was developed to eliminate the potential for cone formation (non-compendial).

USP Apparatus 3-Reciprocating Cylinder

The vessels are represented by cylindrical flat-bottomed glass of about 325 ml capacity. The glass of the reciprocating cylinders have inert fittings and screens are placed at the top and bottom of the cylinders. It has a reciprocating agitation of 5 to 35 dips/min, through 10 cm vertical distance. The dosage form is placed in the cylinder, and the cylinder moves horizontally to different rows of vessels. Drug products usually tested using this apparatus are: solid dosage forms (mostly non-disintegrating)-single units (e.g. tablets), multiple units (e.g. encapsulated beads); it has originally been used for extended release products,

particularly beads in capsules. It also generates fractional dissolution results. The advantages of this apparatus are: that is can be programmable to run dissolution in different media and at different speeds at various times and that one can attempt to simulate pH changes in the GI tract e.g. pH 1, pH 4.5, pH 6.8. The disadvantages are that it is not suitable for dosage forms that disintegrate into small particles, surfactants can cause foaming, it has a small vessel volume and media evaporation can occur for tests of long duration.

USP Apparatus 4 - Flow through cell

There are various dimensions/designs for the cells that form the flow through cell apparatus (compendial 12 mm, 22.6 mm diameters). The flow rates 4 , 8, 16 mL/min (compendial, alternative: 2-32 ml/min). The operation can be: open system- continuous flow or closed system - recirculate media.

The media is changed by exchanging reservoirs. This generates fractional dissolution results. Drug products tested using this apparatus are: solids - tablets, capsules, soft gelatin capsules, implants, powder, granules, semi solids - suppositories, ointments, liquids- suspensions.

The disadvantages of this apparatus are: limited experience with use of the apparatus and the fact that the pump precision influences the results. The advantages are that: volume of media is not limited and so it is suitable for poorly soluble drugs and it also has gentle hydrodynamic conditions.

USP Apparatus 5, 6 and 7 are described in the general chapter <724> Drug Release which includes a sub-section for Transdermal Delivery Systems, including Apparatus 5, 6 and 7 procedure and interpretation for each apparatus. The European Pharmacopoeia includes chapter 2.9.4 "Dissolution Test for TransdermalPatches", a disk Assembly Method (using stainless steel disk assembly corresponding to App. 5), Cell Method (using extraction cells) and Rotating Cylinder Method (using stainless steel cylinder corresponding to App. 6). The Japanese Pharmacopoeia has no methods described for transdermal delivery systems.

USP Apparatus 5 -Paddle Over Disk

It uses paddle and vessel assembly from Apparatus 2 with the addition of a stainless steel disk assembly, a temperature of 32°C, speed: typically 50 rpm. The drug products tested are transdermal patches.

USP Apparatus 6 -Cylinder

It uses vessel assembly from Apparatus 1 but replaces basket and shaft with a stainless steel cylinder stirring element. It functions at a temperature of 32°C, the dosage unit is placed on the cylinder with release side out. The drug products tested are reservoir transdermal patches.

USP Apparatus 7 -Reciprocating Holder

It is similar to Apparatus 3 but with different dimensions. It functions at a temperature of 32°C (for transdermal dosage forms). It can have various devices to hold transdermal patches, tablets, capsules, implants. The working speed is 20-50dpm.(Way T.,2013)

The most widely used Apparatus are 1 and 2, aqueous media with pH 1.2-6.8, with surfactants (lauryl sodium sulfate (SLS/LSS), dodecyl sodium sulfate (SDS/DSS), labrasole, polysorbate 20, polysorbate 80, byrj-35, triion X – 100, N,N-dimethyldodecylamide-N-oxide) for poorly soluble products. The usual volume varies 500-1000 ml, at a temperature of 37 ± 0.5°C, dissolution time 15 minutes required for the dissolution of 85% of the tested product. (C Noory et.al.,2000)

Non compendial Methods for quality control tests

In order to be able to deal with the daily challenges of current practice in the pharmaceutical industry, many compendial methods have been adapted to different needs. Among these we mention:

1. Intrinsic Dissolution Apparatus

Intrinsic dissolution is defined as the dissolution of a pure drug substance from a specified constant surface area. A special punch and die is used to compress pure drug substances into a disk or tablet. The disk is placed into a special holder that allows only one flat surface to come in contact with a dissolution media at any time during the test. Intrinsic dissolution data is generally used in drug screening but can provide helpful solubility information for method development.

2. Suspended Basket Method

Each tablet is placed in a basket, tablet cover is placed horizontally, the evaporation cover is mounted, the bottom of the basket is placed at 1 cm above the top of the paddle; the paddle operates at 50 rpm.

Using Media Dissolution vessels of larger volumes (up to 4000 ml) or smaller (100 ml)

3. Mini-Paddle Apparatus

4. Mini-Basket Apparatus

5. Rotating Bottle

It was developed in 1958, it used vessel volumes of 500, 250, 100, 50, 15, 4mL. It was used for long term extended release testing, tablets, capsules, suspensions, implants.

6. Franz Cell

It is an apparatus with vertical diffusion cell, proposed for inclusion in USP together with the enhanced Agilent Cell.

7. USP 2 Apparatus modified for smaller volumes.

Quality control tests – oral solid forms (capsules and tablets)

Quality control tests represent a significant part of the quality assurance process which is meant to assure a high standard for the pharmaceutical product.

For oral solid forms quality control tests involve verifying aspect (dimension, shape, thickness), organoleptic properties (color, smell), mass uniformity, content uniformity. Compendial methods include: mass uniformity, disintegration test, dissolution test, assay. Non compendial tests include: determination of hardness (using Monsanto apparatus, Pfizer apparatus, Stong Cobb, Eweka, Casburt) and friability.

Soft gelatin capsules

Quality control tests for soft gelatin capsules involve the assessment of aspect, filling rate, content uniformity, mass uniformity, disintegration test, dissolution test, microbiological tests, active pharmaceutical ingredients assay.

Disintegration test

Dissolution media is usually water, hydrochloric acid 0.1 M or artificial gastric juice. USP Apparatus 2-Paddle is usually used in cases in which the capsule's content does not affect the paddle. The sample is analyzed after 30 minutes. If the capsules do not correspond, the procedure is repeated for six more capsules without the paddle (if the capsules adhere to the paddle, hindering the test). The test is considered adequate if all six capsules disintegrate in 30 minutes.

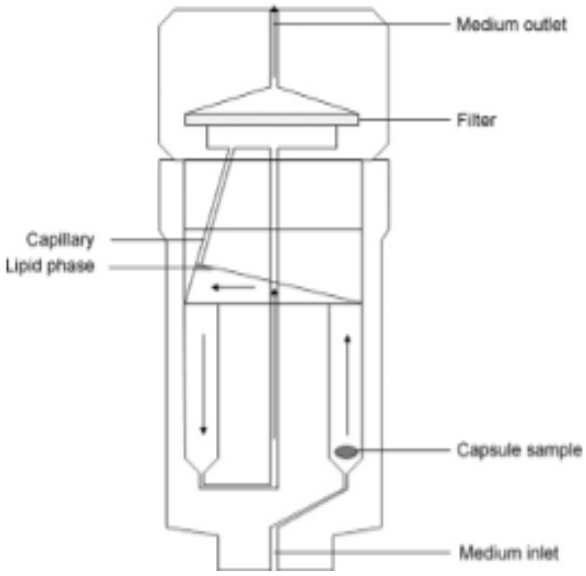

Fig. 1 Schematic view of Flow-Through Cell designed for lipid-filled soft gelatin capsules

The disintegration test assesses the rate of weathering of the pharmaceutical form in time, in a set disintegration media. For dietary supplements, USP-NF states the specification limits must be according to their individual monographs. Apparatus A is mostlyused (described in general chapter <701> Disintegration) for capsules or tablets up to 18 mm in length. Apparatus B is also used, but for longer capsules/tablets. Chapter <701> is harmonized with the European and Japanese Pharmacopoeia. Apparatus A consists of a 1000 ml vessel, 6 tubes provided with sieves at the bottom part.

Rupture test is a specific test applied to soft capsules. USP Apparatus 2 (Paddle) is used, aqueous media, volume of 500 ml, rotation speed of 50 rpm for 15 minutes. The test can be performed with adding papaine (resulting in an activity of NMT 550,000 Units/L) or bromeline (NMT 30 gelatin-dissolving units (GDU)/L of dissoluion media)

Dissolution test

Due to reticulation during the dissolution test for soft capsules a pellicle might appear. In order to prevent this phenomenon enzymes are added to the media according to the pH of the dissolution media:

– if pH<4.0, pepsin is added resulting in an enzyme activity of NMT 750,000 Units/L of the dissolution media

– if 4.0<pH<6.8, papaine is added resulting in an enzyme activity of NMT 550,000 U/L of the dissolution media or bromelin resulting in an enzyme activity of NMT 30 GDU/L of the dissolution media

– if pH>6.8, pancreatin is added resulting in a protease activity of NMT 2000 U/L.

In the case of dissolution media that contain surfactants or other agents that distort enzimatic activity, a pretreatment is used. Currentry, Apparatus USP 2 or USP 1 is used for the dissolution test of soft capsules. Apparatus USP 3 can also be used, having the advantage of a more efficient mechanical agitation, but the dissadvantage of a small dissolution media. Apparatus USP 4 is also used for soft capsules with liquid content.

Non compendial apparatus have been used for the dissolution test of soft capsule. The Flow-Through cell Apparatus with dual cell (also recommended for dissolution test of lipophilic suppositories) (Sree C. Lakshmi et. al, 2013) has been designed in order to overcome the disadvantages of the compendial one. Due to the low density of the lipophilic component of the soft capsules filled with oils, this component rises at the surface of the dissolution media after the disintegration of the capsule. When the lipophilic component is at the superior triangular part of the left cell, it stops at this level and in this way the dissolution media extracts flow through the active substance from the lipophilic component.

The Pillay & Fassihi model is a non compendial apparatus with biphasic dissolution media. The organic phase is used to extract the lipophilic component of soft capsule. This apparatus is an adjustment of the USP Apparatus 2 (Paddle) using also anti flotation devices.

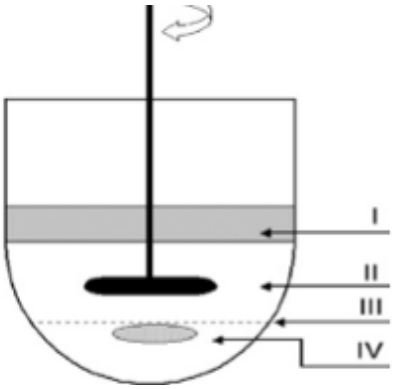

Fig. 2 Schematic view of Apparatus for the dissolution testing of lipid-filled soft gelatin capsules (I=organic phase e.g. 100ml, II=aquous phase, III=ring/mesh, IV=position of capsule, Sree C. Lakshmi et al,2013)

Tablets

The *in vitro* dissolution test is an important quality parameter for tablets and can be used as a predictability criteria for the determination of the bio availability of an active substance in early stages of drug formula development. Dissolution speed is the most important factor regarding future bio-availability of a substance and there is an increasing tendency in replacing the disintegration test with this parameter.

Compendial quality control for tablets is performed using thermostatic devices based on forced convection in non-sink conditions (USP Apparatus 1 and 2, Eur. Ph) and in sink conditions (Flow through cell-USP Apparatus 4). Non compendial methods differ by design and enhancement. Among these we mention the Erweka apparatus with automatic sampler. This device can be connected on/off-line to a HPLC-UV detector. The Erweka-Zeiss apparatus uses optic fibers for substance assay during determination. For poorly soluble active substances dissolution, a multi-compartment apparatus was developed, having different pH value in each compartment.

According to a recent FDA Guide (FDA, 2015a. Waiver of in vivo bioavailability and bioequivalence studies for immediaterelease solid oral dosage forms based on a biopharmaceutics classification system. Guidance for Industry (draft guidance), Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2015, for immediate - release solid oral dosage forms, Biopharmaceutics, Revision 1) combining BCS classification features with *in vitro* release profiles studies could lead to the possibility of choosing a dissolution media that would mimic the *in vivo* result (especially for BCS class 1 and 3). Another Guide from the same year, (FDA, 2015b. Dissolution testing and specification criteria for immediate-release solid oral dosage forms containing biopharmaceutics classification system class 1 and 3 drugs. Guidance for Industry (draft guidance), Food and Drug Administration, Center for Drug Evaluation and Research (CDER), August 2015, Biopharmaceutics) states that although the digestive tract is complex and the conditions cannot be reproduced, using Apparatus USP 1 or 2, at a rotation speed of 100 rpm (Apparatus USP 1) and 75 rpm (Apparatus USP 2) have proved to be discriminatory. Also, in order to mimic gastric volume, the dissolution media volume was reduced from 900 to 500 ml. (Symonds J. et al, 2016)

Differential scanning calorimetry (DSC)

It is a technique based on the measurement of the thermic properties of a substance. Calorimetry is the only method that can directly measure the enthalpy of the process and also establish a connection between temperature and the physical characteristics of a sample.

The differential scanning calorimeter is a device that measures the way in which the physical properties of a sample vary as the temperature rises and as a function of time. In other words, this instrument determines temperature and heat transfer associated with the sample's transition as a function of time and temperature. It measures the heat exchanged by a sample versus a reference subjected to the same thermic conditions. Based on the principle of functioning the differential scanning calorimeters can be classified as:

- heat flow calorimeters – recording the temperature difference between reference and sample
- heat compensation value calorimeters-transforming the difference of temperature in caloric power needed to compensate thermic equilibrium between reference and sample.

Although both models use measurement of temperature recorded by sensors, the differential heat flow from the oven to the sample and reference is measured by different ways.(P. Gill, et al, 2010).

DSC has different applications among these we mention the study of interaction between active substance and excipient. Yuan et al. Has developed a method of determination for the performance of active substances encapsulated in lipophilic nanospheres. (Yuan H. At al,2008)

Determining the active substance quantity

UV-Spectroscopy is the most common method used to determine the active substance quantity. The samples are filtered, transferred into a vessel, measured using UV spectroscopy directly or after HPLC separation. In some cases flow through cell is used, coupled with UV-spectroscopy device, the sample is transferred using a pump and the testing is done in real time. (Wang Q. et al, 2006)

Classification of quantification methods

1. Spectrophotometric

- UV-VIS

If excipients can alter the determination, multi-partitioned analysis is used.

- Fluorescence and Chemiluminescence

First method is used for fluorescent (natural or induced) substances. The second one has a higher sensitivity with a more advantageous detection limit, is used for substances lacking a chromophore group.

- IR/Raman

Fourier-transform infrared spectroscopy (FTIR), close infrared and Raman spectroscopy require high analyte quantities.

2. Chromatographic

- HPLC

Analytes are separated in a stationary phase (column) and a mobile one, retention time is recorded.

- Capillary electrophoresis

It is used for ionic particles that cannot be separated using the previous method.

3. Mass spectroscopy

Particles with electric charge are characterized by mass/charge ratio. Mass spectrometer is coupled with HPLC or GC (gas chromatography). For ionization, different techniques can be used: electrospray, chemical ionization at atmospheric pressure, field ionization, rapid atom bombing.

4. Potentiometric

The chosen method used for assay is based on the chemical characteristics of the tested sample. Another key element is dissolution media. For poorly soluble active substances, surfactants are generally added and for HPLC dosing a pre-treatment is needed because of the interference of surfactants in the assay.

Performance tests

The performance of a pharmaceutical product can be defined as the measure in which the active substance is released from the pharmaceutical form (*in vitro*), extrapolating to the effect of the active ingredient in the living organism (*in vivo*).

The release profile of an active ingredient from a pharmaceutical form is obtained through the *in vitro* dissolution test. The difference between the dissolution test seen as a quality control test and the dissolution test considered a performance test is that in the latter case, the determination involves comparison between the sample's behavior in dissolution media at pH values of 1.2, 4.5, 6.8 and the reference behavior in the same media. Quality control test involves only one determination at a particular pH value. The in vivo performance test involves a bioequivalence test (Shargel et al, 2010).

Compendial methods for soft gelatin capsules- FIP (International Federation of Pharmaceutical Sciences**)-AAS (**American Association of Pharmaceutical Scientists**) recommendations**

Compendial methods are generally recommended by the two association as first choice methods for quality control/performance tests.

Table 3
FIP/AAPS Recommendations

Dosage form example	Release meathod
Oral solid dosage form	Basket Apparatus, Paddle Apparatus, Reciprocating cylinder or Flow-Through Apparatus
Oral suspension	Paddle Apparatus
Oral disintegrating tables	Paddle Apparatus or Disintegration method
Chewable tablets	Basket Apparatus, Paddle Apparatus, Reciprocating cylinder
Powders and granules	Flow-Through Apparatus (powder or granule sample cell)
Thin dissolvable films	Basket Apparatus or Disintegration method
Chewing gum	Special Apparatus (Ph.Eur)
Dermal delivery system (paches)	Paddle over disk
Topical (semisolid dosage forms)	Franz Cell diffusion system
Suppositories	Paddle Apparatus, modified Basket Apparatus or dual chamber flow-through cell Apparatus
Microparticulate formulations	Modified flow-through cell Apparatus
Implants	Modified flow-through cell Apparatus
Aerosols	Cascade impactor

Adaptations to the compendial methods can be approved if the necessity and efficiency of the new methods can be proved.

Soft gelatin capsules can have hydrophilic or lipophilic content. For the latter case, surfactants or other emulsifiers are commonly used. USP-NF recommends the use of Apparatus 2 (Paddle) with small amounts of surfactant added if necessary. If the liquid content of the capsules is hydrophilic surfactants are not needed. Floatability can be a problem for the dissolution Apparatus 2. Formulations containing emulsifiers can suffer separation of phases

phenomenon at the liquid-vessel-air inter-phase, or the sample might adhere to the vessel or paddle.

Rupture test is used to determine the performance of soft gelatin capsule. If the active ingredient is dissolved or suspended in a lipophilic matrix a pre-treatment us needed, usually enzymatic: e.g.: pancreatin. Rupture time of the capsule is measured.

Besides USP Apparatus 2, FIP-AAS recommends the usage of Apparatus 1 (Basket), which has the advantage of absence of floatability problem. Also, Apparatus 3 is used (having the advantage of a more homogeneous media, but the disadvantage of small dissolution media volume) and Apparatus 4. In the case of surfactants presence in lipophilic capsules enzymatic treatment is used to simulate digestion if this is a limiting factor for dissolution and *in vivo* absorption (Brown K. et.al, 2011).

Modified flow through cell apparatus- dual cell (recommended in Ph.Eur.2.9.3-6 for the dissolution profile of lipophilic suppositories) is considered a proper test for soft capsules with liquid content also. It can function in closed or opened system (this aspect is important in the case of self-emulsifying systems). A possible drawback is the fact that it can become opacified during tests. (Siewert M et.al, 2003)

Coenzyme Q10 release profiles

Coenzyme Q10 is a pervasive lipophilic molecule. It was discovered by professor F. Crane and research colleagues at the University of Wisconsin-Madison Enzyme Institute in 1957. A year later, dr. K. Folkers and colleagues at Merck discovered it's exact chemical structure. In 1961 P. Mitchell proposed the electron transport chain (which includes the vital proton-motive role of CoQ10) and he received a Nobel prize for the same in 1978. In the mitochondrial internal membrane the electrons coming from NADH and succinate pass through the electron transport chain which is then reduced to water. The electron transfer results in H+ formation in the membrane; also, a proton gradient is generated along the membrane, which is used by ATP-synthetase to generate ATP. Coenzyme Q10 acts as an electron transporter from the enzymatic complex I and II to III in this process (recent studies show the concomitant role of vitamin K2). In 1972 Gian Paolo Littarru and Karl Folkrs separately demonstrated that Coenzyme Q10 deficiency is present in heart failure.

Coenzyme Q10 is an important antioxidant, together with Vitamin E it protects lipoproteins by preventing oxidation. It also has an important role in preventing atherosclerosis, due to the redox function; it has the ability to exchange electrons in the redox cycle between ubiquinol - the reduced form, and ubiquinone -the oxidized form. The antioxidant role of Coenzyme Q10 is due to it's ability to neutralize free radicals. In neurodegenerative dysfunctions Coenzyme Q10 prevents the formation of lipid-peroxide radicals. Natural sources: sardines, pork, beef, olives, broccoli, butter. (Littarru GP, et.al, 2011).

In vitro dissolution profiles of Coenzyme Q10

Coenzyme Q10 is a large lipophilic molecule, with poor solubility in water and good permeability. (Class II BCS). Because of these properties, there are numerous attempts mentioned in literature to increase Co enzyme's Q10 solubility and bio-availability in oral solid pharmaceutical forms. Among these we mention:

– solubility increase by adding surfactants: lecithin, polysorbates;

– powder obtained by freeze-drying of a Co enzyme q10 emulsion in a modified polysaccharide matrix (Chen C. et al 2005);

– particle size reduction (80 nm, 120 nm, 400 nm, 700nm) with noncrystalline formation and addition of Tween 80 surfactants (Jiao Sun et al. 2012);

– micelle or liposome formulations

– cyclodextrin formulations

– self-emulsifying systems

Are systems composed of a lipophilic phase, surfactants and co-surfactant. Prabagar Balakrishnana et. al., 2009 described in the article *Enhanced oral bio-availability of Coenzyme Q10 by self - emulsifying drug delivery systems*, the components of the self-emulsifying system: lipophilic phase (Labrafil M 1944 and Labrafil M 2125), Labrasol surfactant and co-surfactant (Lauroglycol FCC and Capryol 90). Particle size (240 nm) and zeta potential were measured as well as self-emulsifying time. The optimal formulation proved to be 65% (v/v) Labrasol, 25% (v/v) Labrafil M 1944 CS and 10% (v/v) Capryol 90.

Differential scanning Calorimetry can provide valuable information on the interaction between the active pharmaceutical ingredient and the excipients. Farboud et al. published an article in 2011 in International Journal of Nanomedicine, on the Co enzyme q10 nanoencapsulation performance.

Palamakula A., 2004 published an article on Coenzyme Q10 and limonen in self-emulsifying systems in which dissolution profile was determined using Apparatus USP 2 (paddle); also the system was analyzed using DSC, zeta potential measurement and HPLC-UV dosing. Coenzyme Q10, R-(+)-limonen and less than 50 % Cremophor EL formulations had the best performance test results.

In Japan Coenzyme Q10 has been used as a dietary supplement since 2001. Kettawan A. published in 2007 a study on different pharmaceutical oral forms - the release profiles of Coenzyme Q10 from capsules, soft gelatin capsules, tablets, granules and jelly formulations. The following quality control tests were included: disintegration test (according to J.P.XV), content uniformity, assay (HPLC-ECD). Some samples tested did not disintegrate in 1 hour at 37°C.

Lunetta et al.2008 conducted a study to prove the efficiency of HPLC-UV in Coenzyme dosing from capsuls, soft gelatin capsules and chewable gums.

Result evaluation

Graphic representation as curves of *in vitro* release profiles (dose in time) are used for methods based on stirring (Apparatus USP 1 and 2). For flow through cell apparatus the active substance cumulatively released is graphically represented as a function of time.

Curve parametrization is the process of describing a dissolution profile with the use of constants.

- empiric parametrization – is independent from the curve model – the important parameters are: quantity, concentration (active pharmaceutical ingredient dissolved in time – t), time needed for a

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certain percentage of the API to be dissolved, dissolution speed;

- function parametrization – dependent of curve model – the important parameters are the lows of time: zero-order kinetics, first-order kinetics, square and cubic root low;

When dissolution test is used as a quality control test, the recorded parameters are quantity of dissolved active pharmaceutical ingredient and time. In this particular case, the kinetic model does not play an important role, quantitative determination is sufficient. Empiric parameters have the disadvantage that in order to obtain a more accurate description of the dissolution curves, much data is needed. Function parametrization require notion on dissolution function. In most of the cases, a dissolution curve follows the lows of first-order kinetics only for a certain time interval.

CONCLUSIONS:

Dietary supplements are being used by an ever increasing population, at all ages and together with other medicines. There is a rising need for the regulation to assure uniformity of content and quality of ingredients in order to address heath issues.

REFERENCES:

Anitha Palamakula, Biopharmaceutical classification and development of limonene - based self - nanoemulsified capsule dosage form of coenzyme Q10, Submitted to the Graduate Faculty of Texas Tech University Health Sciences Center in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY, May 2004: 123-125

C Noory et.al., Dissolution Technologies: 7(1): 16-18, 2000.

C. Sree Lakshmi, A.V. Badarinath, An Updated Review of Dissolution Apparatus for Conventional and Novel Dosage Forms, International Journal of Pharma Research & Review, July 2013; 2(7):42-53

Chen Shaw T., 2011, Regulation of resherch: Is it a drug trial or a supplement trial? Fitoterapia 82(2011), 14-16

Chetan Amrutkar, Kishor Salunkhe, Sanjay Chaudhari, STUDY ON SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM OF POORLY WATER SOLUBLE DRUG ROSUVASTATIN CALCIUM, World Journal of Pharmaceutical Research, Vol 3, Issue 4, 2014, 2137-2151

Committe on the Framework for Evaluating the Safety of the Dietary Supplements, National Research Council, National Academies Press [2005], Dietary Supplements: A Framework for Evaluating Safety

Committe on the Framework for Evaluating the Safety of the Dietary Supplements, National Research Council, National Academies Press [2002], Dietary Supplements: A Framework for Evaluating Safety

Complementary and Alternative Medicine in the United States, Institute of Medicine of the National Academies, The National Academies Press, [2005]

Cynthia K. Brown, Horst Dieter Friedel, Amy R. Barker, Lucinda F. Buhse, Susanne Keitel, Todd L. Cecil, Johannes Kraemer, J. Michael Morris, Christos Reppas, Mary P. Stickelmeyer, Chikako Yomota, and Vinod P. Shah, FIP/AAPS Joint Workshop Report: Dissolution/In Vitro Release Testing of Novel/Special Dosage Forms, AAPS PharmSciTech, April 2011

Cynthia K. Brown, Horst Dieter Friedel, Amy R. Barker, Lucinda F. Buhse, Susanne Keitel, Todd L. Cecil, Johannes Kraemer, J. Michael Morris, Christos Reppas, Mary P. Stickelmeyer, Chikako Yomota, Vinod Shah, FIP/AAPS Joint Workshop Report: Dissolution/In Vitro Release Testing of Novel/Special Dosage Forms, Indian J Pharm Sci. 2011 May-Jun; 73(3): 338–353.

Dumitru Lupuleasa, Iuliana Popovici - Tehnologie farmaceutica, ed. A 3-a rev-Iasi Polirom, 2011 3 vol: 252,245-245, 2001-2009: 554-556

Effat Sadat Farboud, Saman Ahmad Nasrollahi, Zahra Tabbakhi, Novel formulation and evaluation of a Q10-loaded solid lipid nanoparticle cream: in vitro and in vivo studies, International Journal of Nanomedicine 2011:6 611–617.

Emmanuel Scheubel. Predictive in vitro dissolution tools : application during formulation development. Pharmacology. Universit´e d'Auvergne - Clermont-Ferrand I, 2010

Ensuring the quality of Dietary Supplements, 2016, USP-U.S. Pharmacopeial Convention, OTH430F_2016-02

Erika Stippler, USP Pharmacopeial Education, Dissolution: Theory and Best Practices,2011

Gabriela Garban, Nicoleta Florescu, Suplimentele Alimentare – Ministerul Sănătăţii - Institutul Naţional de Sănătate Publică Ghid, Ediţia 1 – 2013, 5-14

Gerard Flintoaca, Suplimente alimentare: Legislatie, Autoreglementare si Etica in publicitate, 2016

Hamishehkar H, Ranjdoost F, Asgharian P, Mahmoodpoor A, Sanaie S., Vitamins, Are They Safe?, 2016 Dec;Advanced Pharmaceutical Bulletin, 6(4), 467-477

Hu F-Q, Zhang Y, Du Y-Z, Yuan H. Nimodipine loaded lipid nanospheres prepared by solvent diffusion method in a drug saturated aqueous system. Int J Pharm 2008;348:146–152

Jiao Sun, Fan Wang, Yue Sui, Zhennan She, Wenjun Zhai, Chunling Wang, and Yihui Deng,Effect of particle size on solubility, dissolution rate, and oral bioavailability: evaluation using coenzyme Q10 as naked nanocrystals, International Journal of Nanomedicine 2012; 7: 5733–5744.

John C. Umhau, Keva Garg and Albert M. Woodward, Dietary Supplements and Their Future in Health Care: Commentary on Draft Guidelines Proposed by the Food and Drug Administration, 2012, Antioxid Redox Signal. 2012, 16, 461–462

Kettawan A, Kunthida C, The Quality Control Assessment of Commercially Available

Coenzyme Q10-Containing Dietary and Health Supplements in Japan, J.Clin. Biochem. Nutr. 2007 Sep; 41(2): 124–131

Leon Shargel,Isadore Kanfer, Generic Drug Product Development: Specialty Dosage Forms, Informa Healtcare USA, 2010: 3-4

Littarru GP, Lambrechts P. Coenzyme Q10: multiple benefits in one ingredient. OCL 2011 ; 18(2) : 76-82. doi : 10.1684/ ocl.2011.0374

Martin Siewert, Jennifer Dressman, Cynthia Brown, Vinod Shah, FIP/AAPS Guidelines for Dissolution/In Vitro Release Testing of Novel/Special Dosage Forms, Dissolution Technology, 2003 Febr;6-15

Melethil Srikumaran, 2006, Proposed rule: Current good manufacturig practice in manufacturing, packing, or holding dietary ingredients or dietary supplements, Life sciences (78) (2006), 2049-2053

Mister S., Hathcock J, 2012, Under the law, FDA must grant different standards for new dietary ingredients and food additives, Regulatory Toxicology and Pharmacology, 62 (2012) 456-458

Mohd Yasir , Mohd Asif, Ashwani Kumar, Abhinav Aggarval, Biopharmaceutical Classification System: An Account, International Journal of PharmTech Research, Vol.2, No.3, pp 1681-1690, July-Sept 2010.

N. Subramaniana, P. Chandra sekarb, S. Abimanyuc, S. P. Sharavanand, R. Gayathrie, A Meenakshif, M. Nithyakalyanig, IMPROVED ORAL DELIVERY OF ROSUVASTATIN BY USING SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM , Advanced Materials Research Vol. 678 (2013) pp 286-290

Pavan Ram Kamble, Karimunnisa Sameer Shaikh, Pravin Digambar Chaudhari, Application of Liquisolid Technology for Enhancing Solubility and Dissolution of Rosuvastatin, Advanced Pharmaceutical Bulletin, 2014, 4(2), 197-204

Perrut M, Jung J, Leboeuf F. Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes Part 1: Micronization of neat particles. Int J Pharm 2005; 288:3-10.

Pooria Gill, Tahereh Tohidi Moghadam, and Bijan Ranjbar, Differential Scanning Calorimetry Techniques: Applications in Biology and Nanoscience, Journal of Biomolecular Techniques 21:167–193, 2010

Prabagar Balakrishnana, Beom-Jin Leeb, Dong Hoon Oha, Jong Oh Kima, Young-Im Leea, Dae-Duk Kimc, Jun-Pil Jeec, Yong-Bok Leed, Jong Soo Wooa, Chul Soon Yonga, Han-Gon Choia, Enhanced oral bioavailability of Coenzyme Q10 by self-emulsifying drug delivery systems, International Journal of Pharmaceutics 374 (2009) 66–72

Qingxi Wang, Decheng Ma, and John P. Higgins, Analytical Method Selection for Drug Product Dissolution Testing, Dissolution Techniques, 2006 August; 6-12

Scheubel Emmanuel, Lindenberg Marc, Beyssac Eric, Cardot Jean-Michel, Small Volume Dissolution Testing as a Powerful Method during Pharmaceutical Development, Pharmaceutics 2010, 2, 351-363

Sharon R. Akabas, Gretchen Vannice, John B. Atwater, Tod Cooperman, Richard Cotter, Lisa Thomas, Quality Certification Programs for Dietary Supplements(2016), Journal of the Academy of Nutrition and Dietetics, 1370-1379

Simons Juliet, Elder David, Dissolution testing in the moderm world, European Pharmaceutical Review VOLUME 21 ISSUE 1 2016;42-45

Vinod P. Shah, Role of Dissolution in regulating pharmaceutical products, 2nd MENA Conference on Bioequivalence, Biowaivers, Bioanalysis and Dissolution Amman, Jordam, 2015

Way Terry, The Fundaments of Dissolution Agilent Technologies, 2013