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Implementation of Water Activity as an Alternative Microbiological Method through on A Risk-Based Approach in the Pharmaceutical Industry

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Abstract: The use of alternative microbiological methods as water activity has been encouraged by the United States Pharmacopeia guidelines and local regulatory entities as INVIMA (National Food and Drug Surveillance Institute), since the quantification of water activity can be used as an indicator of the microbiological, physicochemical, and organoleptic stability of a specimen, since low water activity retards autohydrolysis and microbiological growth. Therefore, general chapters <922 and 1112> supports the use of water activity (Aw) below 0.60, along with historical microbiological data, to justify the reduction or elimination of routine microbiological testing (skip-lot testing). It is important to demonstrate that the product's microbiological quality is maintained when water activity is used as a predictive tool. This could be involved correlating water activity levels with historical microbiological data (at least the last 20 batches) to ensure that products with water activity below 0.60 consistently meet the required microbiological specification. This review summarized that water activity could be used as a microbiological predictable tool using a risk-based approach that should include water activity below 0.60, microbiological historical data, and robust microbiological skip-lot testing.

Keywords –*water activity, dew point chilled mirror method, alternative microbiological methods (AMM), risk-based approach*

I. Introduction

The enforcement of groundbreaking microbiological methods has been growing, since they can offer several benefits in execution, monitoring, and automation while improving accuracy, specificity, sensitivity, and precision [1–6]. Additionally, local regulatory entities such as INVIMA (*National Food and Drug Surveillance Institute*) and USP guidelines encourage its implementation as long as a robust validation process has been performed according with the USP demands. Considering that these alternative microbiological methods (AMM) are usually automated systems, they enable a more rapid and efficient response in case of adverse microbiological outcomes [1,2,3,4]. Furthermore, these cutting-edge technologies significantly reduce the microbiological process time, leading to more rapid release of the pharmaceutical products such as tablets, lozenges, and capsules into the market, allowing a significant reduction of company warehousing costs [4–6]. However, their use in the pharmaceutical industry has tended to be delayed, because these new technologies

used to be expensive and time consuming, these issues being the primary obstacles for their adoption [4–6]. Moreover, pharmaceutical regulators used to be overly cautious in endorsing these alternative methods as an integral part of routine product release [4–6].

USP <1112> has been encouraging the pharmaceutical industry to use water activity as an alternative microbiological method (AMM) in products with low water activity levels, because they are potentially not susceptible to being contaminated [7-10]. For instance, solid raw materials (powders), lozenges, tablets, and capsules had reported water activities around 0.30 to 0.50 which makes them excellent target candidates for excluding microbiological tests lot by lot, because at those low water activity levels, it is unlikely that objectionable pathogens, mesophiles, yeasts, and molds would be able to grow on the pharmaceutical article [7,8]. USP chapter 1112, for instance, recognizes new possibilities that allow the implementation of AMMs as a water activity measurement as a direct microbiological assessment for the microbiological bioburden determination in order to exclude routine microbiological analysis batch by batch, which usually takes longer than either performing the method or yielding the final results about the quality status of a pharmaceutical article. However, as is outlined in the USP chapter 1112, the measurements of water activity by itself should not be used as the sole criterion for obviating microbiological test analysis [10]. Therefore, water activity must be used as an integral part of routine product release through a risk-based approach that should include water activity below 0.60, microbiological historical data, and robust microbiological skip-lot testing each 20 lots or 4 months.

II. Literature Review

As outlined in USP general chapters 922 and 1112, water activity (aw), or free water, is the ratio of the vapor pressure of the H_20 in the product and the vapor pressure of pure H_2O at the same temperature [9]. The range of water activity is between 1 (aqueous products) and 0 (dry materials). The free water, or water activity (aw), since it is not a structural part of the chemical formula, plays an important role, since it constitutes the free water potentially available for microorganisms such as bacteria, yeasts, and molds [9,10]. Thus high water activity increases the availability of nutrients to be used by microorganisms, making suitable environmental conditions for gas exchange, generating an osmotically stable environment that allows the secretion of metabolic waste, thus encouraging its proliferation and therefore leading to the spoilage of the pharmaceutical article [7,8], so medications that contain high levels of free water (aw close to 1) will be more susceptible to harboring microbiological growth than pharmaceutical articles with low water activity [1,2].

Currently, there are several devices that quantify water activity either by measuring the water activity directly or by measuring secondary parameters related to the water activity [9]. USP chapter 1112 suggests quantifying water activity using the dew point chilled mirror method(DPCMM), since it generates accurate and reproducible results in an average reading period of 7-10 minutes [7,8,10]. Therefore, pharmaceutical products, because of their hygroscopic features, will enter into equilibrium with the surrounding environment [7-10]. This means that the free water in the product is released as vapor to the surrounding environment until equilibrium is reached in the headspace of the measuring chamber [10]. Once equilibrium has been reached, a dew point is formed over the chilled mirror surface, and this will be equivalent to the free water of the pharmaceutical sample tested. It is important to understand that equilibrium is reached when the pharmaceutical article neither releases nor absorbs moisture from the surrounding environment [7,8,10].

A polished chilled mirror is used as the condensing surface, and it is usually placed above the sample into the measuring chamber [9, 10]. The cooling system is electronically connected to a photoelectric cell, from which light is reflected onto the condensing mirror. An air flow produced by a fan is directed toward the polished chilled mirror until it forms the dew point over the cooled mirror at equilibrium [9,10]. This cooling system helps the sample to come into equilibrium with the surrounding environment much faster. The dew point formed on the mirror is detected by the light beam, which is reflected in a distorted manner onto the photoelectric cell [10]. The dew point temperature will be a direct measure of the equilibrium vapor pressure of pure water, and the sample temperature will be a direct measure of the H_2O vapor pressure in the product [9, 10]. Thus, the

vapor pressure of the water in the product in relation to the vapor pressure of pure water at the same temperature will allow the water activity of the product to be quantified in a reliable and reproducible manner [9, 10].

III. Results: Water activity and microbiological specification in solid pharmaceutical products

As it has previously been shown in various research studies carried out at Coaspharma S.A.S Company, it was demonstrated that based on calibration curves using saturated salts, it was possible to establish the linearity and operating range of the DPCMM [7]. The evidence demonstrates that this alternative automated method yields precise and accurate results (see Table 1). Its ability to remain unaffected by different operational variables such as different operators was evidence of its reliability and stability. Although water activity differences among batches were observed for all the tablets and capsules tested (ANOVA $P<0.05$), those differences corresponded to manufacturing process variations that impacted the water activity status of solid raw materials, tablets, lozenges, and capsules [7, 8]. Moreover, the DPCMM shows a high degree of concordance (SD < 0.01).

As outlined in USP chapter 1112, pharmaceutical products with water activity far below 0.75 are excellent target candidates for obviating microbiological tests, because at these low water activity levels, it is unlikely that objectionable pathogens, mesophiles, yeasts, and molds would be able to grow on the pharmaceutical article [9,10].

Water activity measurement using a validated DPCMM has been carried out in solid pharmaceutical products such as powdered raw materials, lozenges, tablets and capsules. Simultaneously, microbiological assessments as yeast and mold counts, mesophiles counts and pathogens assessment have been performed using the reference standard methods based on plate-count method [7, 8]. As it is depicted in table 1 and 2, for each solid sample tested which has water activity under specification ($aw<0.60$), the microbiological test results for mesophiles (counts < 10 cfu/gram), *E. coli* (absent), yeast and molds (counts < 10 cfu/gram) fit microbiological specification for human use(see Table 2 and 3). Therefore, water activity status could be considered as a reliable measure for microbiological burden at least in the solid sample tested [7, 8].

Table 1. Saturated salt check standards used to build up the calibration curves at 25 ºC. For each standard, 6 replicates were taken in order to calculate mean and standard deviation (SD).

	Water activities measured by Aqualab 4TE				
Standard salt	**13.41 mol/Kg LiCl 0.250**	**8.57 mol/Kg LiCl 0.500**	**6.0 mol/Kg NaCl 0.760**	**2.33 mol/Kg NaCl 0.920**	**Deionizedwater 1.00**
Water activity Average (n=6) Aw^o	0.2498	0.4992	0.7616	0.9231	1.0058
Standard deviation	0.0002	0.0003	0.0005	0.0005	0.0018
Relative standard deviation	0.0777	0.0690	0.0613	0.0522	0.1791
Standard salt (Aw)	0.2500	0.5000	0.7600	0.9200	1.0000
Aw^o-Aw	0.0002	0.0008	-0.0016	-0.0031	-0.0058
RepeatabilityAqualab	0.00039	0.00069	0.00093	0.00096	0.00360
UncertaintyAw^o	0.003	0.003	0.003	0.003	0.003
RepeatabilityAqualab+ Uncertainty	0.00339	0.00369	0.00393	0.00396	0.00660
USP Chapter 922 (Absolute error)	**ABS ERR = Aw^o- Aw ≤** Repeatability Aqualab + Uncertainty**Aw^o**				

Table 2. Water activity status on tablets, capsules, and lozenges.

TABLETS, CAPSULES, AND LOZENGES	Aw (n=9)	Microbialgrowth
CHLORPHENYRAMINE 4 mg TABLETS	0.4412	Absent
METRONIDAZOLE 300mg, NIFUROXAZIDE 200mg CAPSULES	0.5025	Absent
METRONIDAZOLE 600 mg-NIFUROXAZIDE 200 mg CAPSULES	0.4538	Absent
PROPRANOLOL 40 mg TABLETS	0.4546	Absent
AMLODIPINE 5 mg TABLETS	0.4361	Absent
METHOCARBAMOL,IBUPROFENE 500/200mg TABLETS	0.4363	Absent
CELECOXIB 200 mg CAPSULES	0.4418	Absent
AMOXICILLIN 500 mg CAPSULES	0.477	Absent
AZITHROMYCIN 500 mg TABLETS	0.5055	Absent
CIPROFLOXACIN 500 mg TABLETS	0.4151	Absent
FLUNARIZINE 10 mg TABLETS	0.393	Absent
GEMFIBROZIL 600 mg TABLETS	0.4683	Absent
LORATADINE 10 mg TABLETS	0.4496	Absent
AMPICILIN 500 mg CAPSULES	0.4284	Absent
NAPROXEN 220 mg + ACETAMINOPHEN 250 mg + CAFFEINE 65 mg TAB. CAPSULES	0.4272	Absent
PREDNISOLONE 5 mg TABLETS	0.4048	Absent
IBUPROFENE,CAFFEINE 200/30 mg CAPSULES	0.4240	Absent
METHOCARBAMOL 750 mg TABLETS	0.5003	Absent
NITAZOXANIDE 500 mg TABLETS	0.4577	Absent
PIROXICAM 20 mg CAPSULES	0.4971	Absent
HYDROXYCIN 25 mg TABLETS	0.4594	Absent
DESLORATADINE 5 mg TABLETS	0.4565	Absent
KETOCONAZOLE 200 mg TABLETS	0.5305	Absent
GLIBENCLAMIDE 5 mg	0.4318	Absent
SILDENAFIL 50mg TABLETS	0.4701	Absent
METOCLOPRAMIDE 10 mg TABLETS	0.4425	Absent
LOSARTAN POTASSIUM 50 mg TABLETS	0.4073	Absent
MELOXICAM 15 mg TABLETS	0.3387	Absent
MELOXICAM 7.5 mg TABLETS	0.3435	Absent
NITAZOXANIDE 100 mg/5mL POWDER	0.4905	Absent
ETORICOXIB 120 mg TABLETS	0.4479	Absent
AMLODIPINE 10 mg TABLETS	0.4486	Absent
TINIDAZOLE 500 mg TABLETS	0.4444	Absent
ORLISTAT 120 mg CAPSULES	0.4032	Absent
CLOPIDOGREL 75 mg TABLETA	0.2531	Absent
ALBENDAZOLE 200 mg TABLETS	0.4742	Absent
SILIMARINE 150 mg CAPSULES	0.4290	Absent
FUROSEMIDE 40 mg TABLETS	0.4386	Absent
ACETAMINOPHEN 325 mg + N-BUTYL HYOSCINE BROMIDE 10 mg	0.4459	Absent

HIOSCINE N BUTYLBROMIDE10 mg TABLETS	0.4801	Absent
FLUCONAZOLE 200 mg CAPSULES	0.4538	Absent
NAPROXEN 500 mg TABLETS	0.4658	Absent
SECNIDAZOLE 1 g TABLETS	0.4369	Absent
ACETYLSALICYLIC ACID 500 mg TABLETS	0.417	Absent
HYDROCHLOROTHIAZIDE 25 mg TABLETS	0.4387	Absent
ACICLOVIR 800 mg TABLETS	0.4258	Absent
CETIRIZINE 5 mg+PHENYLEPHHRINE 10 mg+CAFFEINE 30 mg+ACETAMINOPHEN 500 mg	0.4427	Absent
DIMENHYDRINATE 50 mg TABLETS	0.3996	Absent
IBUPROFENE800 mg TABLETS	0.4002	Absent
FLUOXETINE 20 mg TABLETS	0.3902	Absent
ENALAPRIL MALEATO 5mg TABLETS	0.4883	Absent
ENALAPRIL 20 mg TABLETS	0.4489	Absent
ROSUVASTATINA 40 mg TABLETS	0.4811	Absent
ROSUVASTATINA 10 mg TABLETS	0.4775	Absent
SERTRALINE 50 mg TABLETS	0.4763	Absent
PIRACETAM 800 mg TABLETS	0.4723	Absent
COLCHICINE 0.5 mg TABLETS	0.4198	Absent
MONTELUKAST 4 mg TABLETS CHEWABLE	0.4523	Absent
PIPEMIDIC ACID 400 mg TABLETS	0.4317	Absent
OMEPRAZOLE 20 mg CAPSULES	0.4208	Absent
NAPROXEN 250 mg TABLETS	0.4324	Absent

*9 replicates were taken in order to calculate water activity average

Table 3. Water activity status on powdered raw materials.

POWDERED RAW MATERIALS	Aw (n=9)	Microbialgrowth
ACETAMINOPHEN	0.5933	Absent
BENZOIC ACID	0.5262	Absent
CITRIC ACID	0.5356	Absent
BLUE LACQUER	0.3778	Absent
STEARIC ACID	0.5329	Absent
GUM FLAVOR	0.5108	Absent
RED LACQUER	0.3679	Absent
FINE GRAIN SUGAR	0.4586	Absent
CROSPOVIDONE	0.053	Absent
MANDARIN FLAVOR	0.2808	Absent
CALCIUM CARBONATE	0.5259	Absent
SILDENAFIL CITRATE	0.519	Absent
HONEY FLAVOR	0.2914	Absent
Colloidal SILICON DIOXIDE	0.4147	Absent
ATORVASTATIN	0.385	Absent
PREGELATINIZED STARCH	0.1387	Absent
LEMON PANEL FLAVOR	0.3001	Absent
HYPROMELLOSE	0.1917	Absent
FUSIDIC ACID	0.4235	Absent
SODIUM TIOSULPHATE	0.4305	Absent
CLOBETASOL PROPIONATE	0.3911	Absent

HYPROMELLOSE	0.2775	Absent
ETHYLPARABENE	0.3599	Absent
VALSARTAN	0.3315	Absent
OPADRY	0.2775	Absent
ACETAMINOPHEN	0.3588	Absent
TUTTY FRUTTY FLAVOR	0.3838	Absent
WHITE OPADRY	0.2720	Absent
SODIUM ACETATE	0.3434	Absent
TERBINAFINE	0.3256	Absent
ESOMEPRAZOLE	0.4838	Absent
GREEN LACQUER	0.4406	Absent
SILIDIFIED CELLULOSE	0.4769	Absent
PIPEMIDIC ACID	0.4657	Absent
BIODRY WHITE	0.4961	Absent
SALICYLIC ACID	0.5014	Absent
EMCOMPRESS	0.4851	Absent
SODIUM STARCH GLYCOLATE	0.1119	Absent
FURAZOLIDONE	0.2965	Absent
CORN STARCH	0.5279	Absent
TADALAPHIL	0.3304	Absent
ORANGE FLAVOR	0.2036	Absent
SUCRALOSE	0.3031	Absent
GLYCYRRHIZATE	0.1285	Absent
ASPARTAME	0.468	Absent
AVICEL	0.2493	Absent
LUDIPRESS	0.4385	Absent
METOCLOPRAMIDE	0.4298	Absent
MICROCRYSTALINE CELLULOSE	0.3728	Absent
MICROCRYSTALINE CELLULOSE	0.3701	Absent
MICROCRYSTALINE CELLULOSE	0.4036	Absent
PROSOLV	0.388	Absent
CALCIUM BICARBONATE	0.5259	Absent
AMOXICILLIN TRIHYDRATE	0.3636	Absent
CROSCARMELLOSE SODIUM	0.0993	Absent
MAGNESIUM STEARATE	0.3699	Absent
POTASSIUM HYDROXIDE	0.0295	Absent
NAPROXENE SODIUM	0.3498	Absent
MICRONIZED PIROXICAM	0.3703	Absent
CLOTRIMAZOLE	0.5578	Absent
DEXAMETHASONE ACETATE	0.4914	Absent
ANHYDROUS DISODIUM PHOSPHATE	0.4539	Absent
ANHYDROUS MONOSODIC PHOSPHATE	0.4515	Absent

*9 replicates were taken in order to calculate water activity average

IV. CONCLUSION

All these water activities calculated for the solid pharmaceutical matrix (Aw<0.60) could be included in a risk-based approach that would put into consideration microbiological test results for at least 20 batches of raw materials, primary packaging, and final products, as well as a validated manufacturing process and a validated cleaning process. Including all those items into a decision tree, it might be possible to avoid microbiological analysis lot by lot and otherwise begin a skip lot microbiological testing scheme. These validation results could help to include water activity as a microbiological indicator to assess the bioburden of mesophyll, yeasts, and molds, as well as objectionable microorganisms such as the *Burkholderia cepacia* complex and *Escherichia coli* in solid raw materials, lozenges, tablets, and capsules with water activity lower than 0.60.

Furthermore, in a study conducted at Coaspharma S.A.S, it was also demonstrated that water activity status may be used as a reliable indicator for the microbiological burden and physicochemical features of pharmaceutical material during holding time studies. This research provides evidence that corroborates that water status may be used as a reliable indicator for the microbiological burden and physicochemical features of pharmaceutical material. However, it is recommended that microbiological and physicochemical tests be included in a skip lottesting supported in a risk-based approach.

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