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Validation of the dew point chilled mirror method for the measurement of water activity on solid pharmaceutical products, testing the effect of different temperatures, sample preparations, and environment exposure times

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Abstract

Objective: The objective of this investigation is to validate the dew point chilled mirror method for quantifying water activity in tablets and capsules, evaluating the effects of different temperatures, sample preparations, and local environmental exposure times.

Key findings: The validation of an acute and precise water activity measurement method has become an important goal in the pharmaceutical industry, because it may help to predict the microbiological bioburden on solid products, since most xerophilic and osmophilic microorganisms are unable to grow at water activity levels below 0.60, to safely reduce the frequency of routine microbiological analysis using conventional methods. For all the solid samples tested, a suitability of method was carried out, considering the sample preparation, environmental exposure times, and different measuring temperatures. Following USP guidelines, essential parameters such as precision (SD < 0.5), accuracy (% recovery in the 95%–105% range), linearity ($R^2 > 0.99$), ruggedness (ANOVA, $P < .05$), robustness, operative range (aw 0.17–1), limits of detection (aw = 0.17), and limit of quantification (aw = 0.25) were met by the dew point methodology.

Conclusion: The dew point chilled mirror method was proven to yield accurate, precise, and robust data, making it an outstanding methodology to be implemented in the pharmaceutical industry for measuring the water activity status in tablets and capsules as a direct assessment of the microbial burden.

Keywords: dew point chilled mirror method (DPCMM); alternative microbiological methods (AMM); rapid microbiological methods (RMM); validation testing

Introduction

The implementation of alternative microbiological methods (AMMs) has been growing over the few last decades, driven by new technological advances since they can offer benefits in execution, monitoring, and automation while improving accuracy, specificity, sensitivity, and precision, and they either reduce the microbiological process time compared with the traditional ones or in fact might completely abolish microbiological tests [1–5]. Additionally, these technologies are more environmentally friendly, because there is a significant reduction in the waste derived from the microbiological reference methods [6–8]. It is noteworthy that AMMs are less labor-intensive, reducing routine processing times, since they automatically generate quality reports, with all the items required in the pharmaceutical industry, thus reducing routine manual data transcription, which risks user mistakes [6–8].

In this way, the dew point chilled mirror method (DPCMM) as an automated system has emerged as an AMM for evaluating the microbiological quality of pharmaceutical articles, in accordance with the free water status that occurs inside the solid pharmaceutical products [9, 10]. Considering that free water availability inside the pharmaceutical articles is a critical factor that strongly limits microbial proliferation, this could be used as a direct measure of the microbial burden, since most xerophilic fungi and osmophilic yeasts are unable to grow at water activity below 0.60 [9, 10].

Nonetheless, USP <1112> has been encouraging the pharmaceutical industry to use water activity as an AMM in products with low water activity levels, because they are potentially not susceptible to being contaminated [9, 10]. For instance, tablets and capsules had reported water activity around 0.30–0.50 which makes them excellent target candidates for excluding microbiological tests, 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 [9, 10]. 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 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 [9].

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Thus according to the USP <1111> requirements' acceptance criteria for the microbiological quality of all solid samples for oral use, such as tablets and capsules, there should be a total count for yeasts and molds of <20 CFUs and an aerobic microbial count of <200 CFUs and the sample should be free of *Escherichia coli* and the *Burkholderia cepacia* complex, to fall under the microbiological specification before the product's release for sale [2, 11]. To reduce this routine microbiological analysis for tablets and capsules using dew point instruments, a risk-based approach should be implemented, and it should include microbiological test results and validation of the manufacturing process [9]. Thus solid pharmaceutical products with water activity levels far below 0.75 could be excellent candidates for reducing or even completely eliminating microbiological tests, keeping the quality standards for microbiological assessment aligned with good manufacturing practices, because at low water activity levels, the product is unable to support microbial growth [12–15].

However, as in the USP, the measurements of water activity by itself should not be used as the sole criterion for obviating microbiological test analysis [9]. Therefore, the microbiological test results of the product for at least the last 20 batches of the final product, including raw materials and primary packaging, should be determined. Besides water activity (aw < 0.60) and microbiological results, a validated manufacturing process, as well as a validated cleaning process, should be considered in the risk-based approach, to efficiently support skipping lot by lot microbiological testing [9].

As outlined in USP chapter 1058, water measurement equipment belongs to group B, so the equipment should be standardized through the construction of a calibration curve with saturated salts with known water activity [16]. This calibration curve allows validating of the new technology in accordance with the requirements of USP chapter 1225 [8]. Thus through the calibration curve, essential validation parameters such as linearity, operative range, precision, accuracy, ruggedness, robustness, limit of detection, and limit of quantification will be tested [7, 8].

According to USP 1225, water activity equipment is classified in validation category III, making repeatability and reproducibility essential validation parameters to be tested to successfully perform the validation of the DPCMM for water activity quantification in tablets and capsules [8], so the main purpose of this validation study was to prove that the dew point chilled mirror method's entire performance meets USP 1225 requirements for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol as representatives of tablets and capsules, although several variables, such as different reading temperatures, different sample preparations, and different sample exposure to the local environment have an effect [8]. First of all, using saturated salts of known aw, a calibration curve was built up both at 25°C and 30°C between the water activity recorded by each saturated salt standard (0.25, 0.50, 0.76, 0.92, and 1) and the water activity measured by the Aqualab 4TE equipment.

For all the solid samples tested, naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol, the suitability of the method was shown, considering the sample preparation (crushed sample vs. whole units), different reading temperatures (25°C vs. 30°C), and sample exposure to laboratory environments (exposed sample for 5 min vs. sample without exposure), to verify if there exist statistically significant differences amongst these different conditions [17–19]. Additionally, validation criteria such as ruggedness (reproducibility), using different batches and users, and precision (repeatability) were established in accordance with the United States Pharmacopeia requirements [17–19]. For each solid sample tested, at least three batches were used, and two users were involved in the sample preparation, as well as in the sample measurement of the water activity [17–19].

Experimental

Materials and methods

Reagents

Primary standard saturated salts with known water activity were used to construct the calibration curve, to determine the operative range and linearity of the DPCMM. The saturated salts used were lithium chloride 13.41 mol/kg ± 0.5% aw = 0.25, lithium chloride 8.57 mol/kg ± 0.5% aw = 0.50, sodium chloride 6.0 mol/kg ± 0.5% aw = 0.76, sodium chloride 2.33 mol/kg ± 0.5% aw = 0.92, and distilled water steam aw = 1.00 ± 0.003. The measuring chamber temperature was set at 25°C and 30°C to perform all the tablets' and capsules' water activity measurements.

The following production samples, naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol, were supplied by Coaspharma Laboratories S.A.S, to perform the dew point validation. For each tablet and capsule tested three batches have been used. For each batch, six replicates were taken to calculate the mean and standard deviation (SD).

Dew point instrument Aqualab 4TE Reagents

An Aqualab 4TE water activity meter dew point instrument and standard salts of known water activity were acquired from supplier Insulab. The system includes a dew point instrument, with a precise temperature measuring chamber, dedicated Skala control software, and a computer. Skala control software stored water activity data on the Amazon web site (AWS). The Skala control software fulfilled CFR 21 part 11, ensuring data integrity and confidentiality. The design qualification (DQ), installation qualification (IQ), operational qualification (OQ), software validation (SV), and performance qualification (PQ) of the system were satisfactorily fulfilled by both user and supplier, according to PDA guidelines.

AQUALAB 4 TE water activity meter (Meter Group, Pullman WA, USA) and supplies were acquired from Insulab S.A.S., Meter group representatives in Colombia.

Suitability of the method

Suitability of the method should be proven for all the tablets and capsules before the validation takes place. Thus, to get accurate and reproducible water activity outcomes, several parameters must be considered, such as sample preparation (whole tablets units vs. crushed tablets) and sample exposure to laboratory environments (sample exposed for 5 min vs. sample without exposure). The local laboratory conditions were relative humidity of 40% and temperature of 25°C.

In all cases, a representative number of tablets and capsules must be placed into the plastic sample cup, taking into consideration that it should not be above 50% of its overall

capability, to avoid mirror chamber contamination during the sample measurement. In the same way, the solid sample should be placed into the disposable cup ensuring that the sample completely covers the button of the plastic cup as well as possible.

For all the tablets and capsules tested, the suitability of the method was performed. Once the efficacy of the dew point instrument for the solid sample was proven, the validation was performed using three different batches and six replicates for each batch. Additionally, two different users were used to calculate the ruggedness for each solid sample tested.

Calibration curve, linearity, and operative range

The plotted values in the calibration curve were fit to a least squares regression, and the coefficient of determination (R^2) was calculated. Data generated by the dew point instrument and the expected real value of the standard saturated salt were plotted in Microsoft Excel to generate calibration curves by plotting the water activity measured by the dew point instrument relative to the known primary salt standard value. In this way, five data points were plotted for the calibration curve (aw = 0.25, aw = 0.50, aw = 0.76, aw = 0.92, and aw = 1). As outlined in USP chapter <922>, calibration curves have a validity of one year. Two different reading temperatures, 25°C and 30°C, were used to construct the calibration curves.

Accuracy

Accuracy is defined as the similarity among the mean test results measured by the Aqualab 4TE equipment compared with the true expected values. As mentioned above, five different check standards (aw = 0.25, aw = 0.50, aw = 0.76, aw = 0.92, and aw = 1) were measured six times (6 replicates), and their average water activity outcomes were tabulated along with their respective true water activity value. The accuracy was calculated through the percentage of recovery at 25°C and 30°C.

Additionally, calibration checkpoints (aw = 0.25 + aw = 0.50), (aw = 0.76 + aw = 1), and (aw = 0.92 + aw = 1) were mixed up in a 1:1 proportion, and six replicate measurements were done using the Aqualab 4TE instrument. The mole fraction for each mixture was calculated and compared with the equipment's measured value. The percentage of recovery measured by the dew point instrument should be between 95% and 105% of the expected value.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined from the calibration curve using the least-squares regression model obtained in the calibration curve, linearity, and operative range section. Thus the LOD was calculated based on the mean of the lowest water activity values obtained from the salt standard 0.25.

The values obtained in the experiments (n = 6) were then averaged to determine the mean and standard deviation values. The LOD and LOQ for the DPCMM were calculated using the following equations: LOD = 3.3 SD/m and LOQ = 10 × SD/m, where SD is the standard deviation and m is the slope of the linear regression obtained for the calibration curve. It is important to take into account that the calibration curve was saved into the Skala control equipment's software. So for the routine uses of the dew point instrument, a verification should be carried out in to verify two points of the calibration curve that preferably fall into the expected water activity range for tablets and capsules. Thus before taking a test sample, a verification reading should be performed using the standard salts lithium chloride 13.41 mol/kg ± 0.5% aw = 0.25 and lithium chloride 8.57 mol/kg ± 0.5% aw = 0.50.

Precision, robustness, and ruggedness testing

For each sample of naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol tested, at least three batches and two users were employed, to assess the reproducibility and repeatability. For each batch, six replicates were taken. Then standard deviation and analysis of variance (ANOVA) were determined. The robustness was assessed for each of the solid samples tested, considering internal instrument parameters such as measuring chamber temperature (25°C vs. 30°C).

Microbiological test

To establish an equivalence of results amongst water activity status and microbiological specifications, parallel and simultaneously microbiological tests have been performed for each sample of naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol tested. Thus, yeast and mold counts have been made using sabouraud dextrose agar (SDA). Similarly, mesophile counts have been performed using tryptic soy agar (TSA). Objectionable pathogens such as *E. coli* have also been tested using Mac Conkey agar.

Results and Discussion

Calibration curve, linearity, and operative range

Calibration curves were constructed from known water activity standard checkpoints (Table 1). Outcomes from each standard point (n = 6) show a high degree of precision

Table 1. Saturated salt check standards used to build up the calibration curves at 25ºC and 30ºC. For each standard, six replicates were taken to calculate mean and SD.

	Water activity measured by Aqualab 4TE					
Standard salt	25°C Mean n = 6	25°C SD	30°C Mean n = 6	30°C SD	Percentage of recovery at 25°C	Percentage of recovery at 30°C
13.41 mol/kg LiCl 0.250	0.2492	0.0002	0.2554	0.0008	99.6800	102.1600
8.57 mol/kg LiCl 0.500	0.4995	0.0002	0.5024	0.0004	99.9000	100.4800
6.0 mol/kg NaCl 0.760	0.7605	0.0003	0.7578	0.0023	100.065	99.7105
2.33 mol/kg NaCl 0.920	0.9227	0.0006	0.9214	0.0015	102.522	100.1522
Deionized water 1.00	1.0041	0.0012	1.0039	0.0012	100.410	100.3900

(repeatability) at both temperatures tested, 25°C and 30°C (SD < 0.003, Table 1). The equivalence of results between saturated salt calibration points and the equipment measure values is a gauge of the linearity of the DPCMM. Indeed, the ability of the dew point method to give linear results according to free water activity availability, keeping the accuracy throughout the operative range, is a key parameter for successfully validating the system, because it shows that the dew point instrument is capable of making accurate measurements from aw = 0.25 to aw = 1 at both temperatures tested, 25°C and 30°C ($R^2 \geq 0.99$, Figure 1).

Quantitation ranges were established from the calibration curves using the five standard check calibration points. So for the dew point instrument, there existed a high correlation ($R^2 = 1$, CC = 1) between the true calibration standard points and water activity measured from aw = 0.25 to aw = 1 at 25°C (Figure 1). Similarly, there existed a high correlation ($R^2 = 0.99$, CC = 1) between the true calibration standard points and water activity measured from aw = 0.25 to aw = 1 at 30°C (Figure 1).

These results demonstrated that the saturated salts are stable at temperatures of 25°C and 30°C, keeping the linearity along the whole operative range. Moreover, at 25°C, the percentage of recovery at checkpoint 0.25 is 99.6800%, at checkpoint 0.50 99.9000%, at checkpoint 0.76 100.0658%, at checkpoint 0.92 102.5222%, and at checkpoint 1.000 100.4100% (Table 1). Similarly, at 30°C, the percentage of recovery at checkpoint 0.25 is 102.1600%, at checkpoint 0.50 100.4800%, at checkpoint 0.76 99.7105%, at checkpoint 0.92 100.1522%, and at checkpoint 1.000 100.3900%. Although the percentage of recovery on comparing both temperatures ranges from 99%–02%, these tiny differences are statistically significant ($P < .05$, ANOVA), showing the strong impact of temperatures on water activity levels.

Suitability of the method for tablets and capsules

For the samples of naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol, the suitability of the method was proven to ensure a successful water activity quantification. Several sample handling preparations were considered to be accurate, with precise outcomes for all the pharmaceutical articles tested. In this way, sample preparation such as crushed tablets vs. whole unit tablets was challenged to address if the percentage of recovery is in the specified 95%–105% range (Figure 2). So the percentage of recovery for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol was 102.9710%, 104.8533%, 102.9710%, 103.8303%, 102.9700%, 102.9710%, and 102.5910%, respectively (Table 2). At the same time, exposure of the samples of naproxen, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol to the local laboratory environment (mean temperature 25°C and RH 40%) for 5 min did not produce water activity changes out of the 95%–105% range (Table 3). The percentage of recovery for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol was 101.1973%, 107.3081%, 101.1973%, 99.5718%, 101.1973%, 101.1973%, and 104.7136%, respectively (Table 3).

However, amoxicillin capsules seem to be more susceptible to surrounding environmental conditions, because the capsule sample absorbed water from the local environment, leading to increases in the water activity levels of more than 105% of the original capsule samples (Table 3). However, the average water activity levels from complete tablet units compared with crushed samples are statistically different (ANOVA, $P < .05$). Similar results have been observed among water activity levels from the samples exposed to the local environment compared with those not exposed (ANOVA, $P < .05$). This inherent variability of these methods must be considered during the selection, development, and validation of the DPCMM.

Thus, taking into account that the percentage of recovery was in the 95%–105% range for the crushed samples as well as for the samples exposed to laboratory conditions for 5 min, the validation was carried out with whole tablets or capsule units, avoiding as much as possible exposure for more than 5 min of the solid sample to the surrounding laboratory environment as the sample was placed into the plastic disposable cup and then into the measuring chamber of the dew point instrument. The duration of the whole process usually takes a brief time of around 50 s to take out tablets and capsules

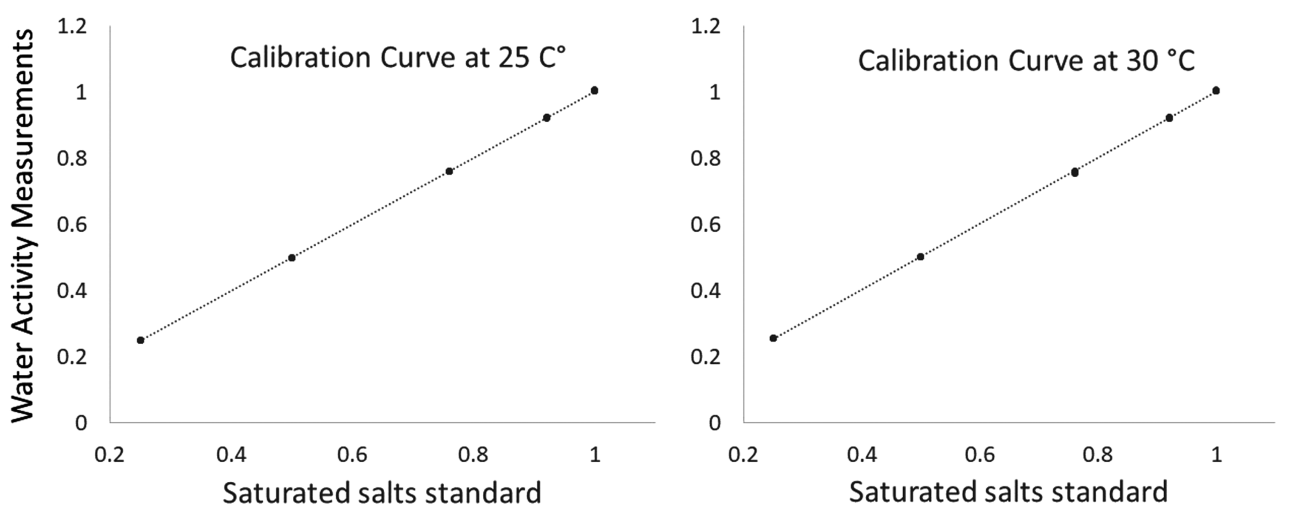

Figure 1. Calibration curves were constructed at 25°C (left) and 30°C (right). Linearity of the dew point chilled mirror method. Data were obtained from experimental measurements using the dew point instrument (axis Y) vs. standard calibration check points (axis X).

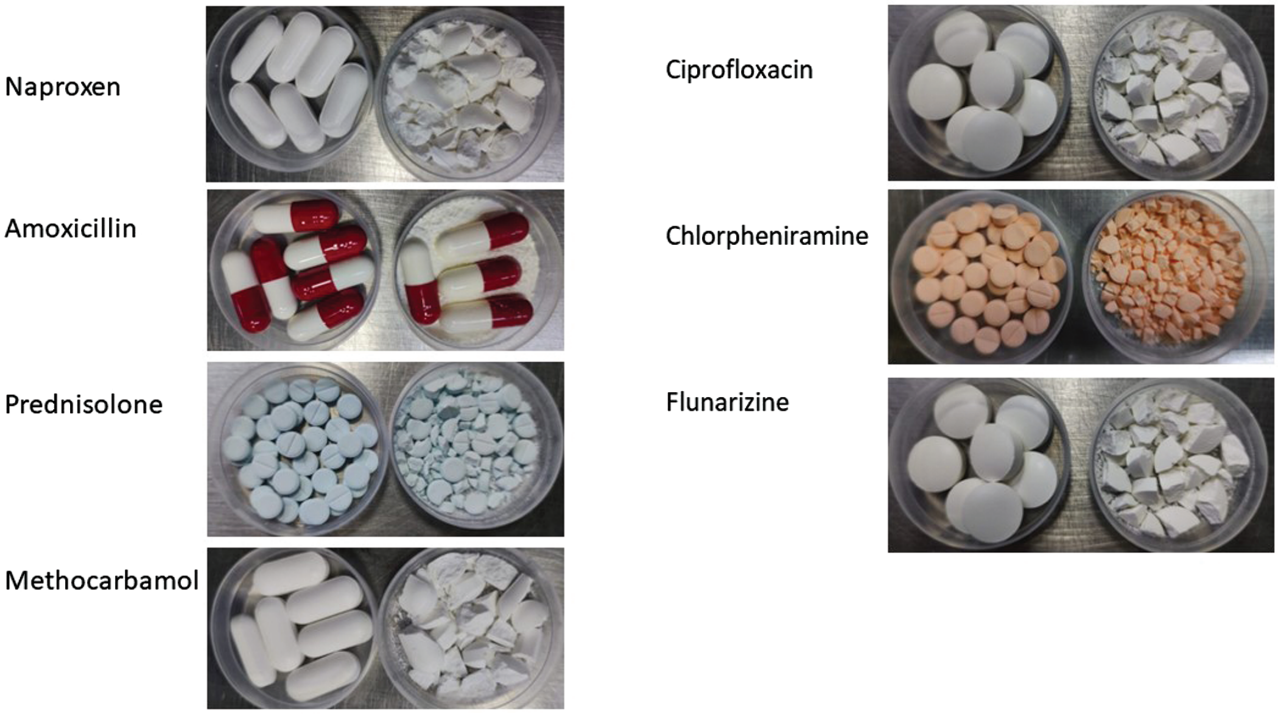

Figure 2. Tested solid samples of naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol in complete units (photo on the left) compared with the crushed tablets (photo on the right).

Table 2. Suitability of the method testing. Effect of different sample preparations (whole tablet unit vs. crushed samples) for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol. For each sample, six replicates were taken to calculate mean and SD.

Tablets and capsules	Crushed sample mean $n = 6$	Whole tablet unit mean $n = 6$	Percentage of recovery
Naproxen	0.4224	0.4300	102.9710
Amoxicillin	0.4852	0.5088	104.8533
Ciprofloxacin	0.4403	0.4545	102.9710
Chlorpheniramine	0.4256	0.4419	103.8303
Prednisolone	0.3899	0.4015	102.9700
Flunarizine	0.3737	0.3715	102.9710
Methocarbamol	0.4728	0.4850	102.5910

Table 3. Suitability of the method testing. Exposure time to the local environment of 5 min for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol. For each sample, six replicates were taken to calculate mean and SD.

Tablets and capsules	Exposed 5 min. Mean $n = 6$	Without exposure. Mean $n = 6$	Percentage of recovery
Naproxen	0.4026	0.4300	101.1973
Amoxicillin	0.4741	0.5088	107.3081
Ciprofloxacin	0.4217	0.4545	101.1973
Chlorpheniramine	0.4438	0.4419	99.5718
Prednisolone	0.3967	0.4015	101.1973
Flunarizine	0.3864	0.3715	101.1973
Methocarbamol	0.4632	0.4850	104.7136

from the blister pack. This sample processing is critical because it will prevent sample water activity changes. However, exposure times of more than 5 min usually led the solid sample to either absorb or lose free water, yielding uncertain readings, and leading to increases in the water activity levels of more than 110% of the original samples (dates not shown). Once the suitability of the method had been met for all the tested tablets and capsules, it was possible to begin with the validation category III, in accordance with USP chapter 1225.

Accuracy

The expected mole fraction value from the saturated standard salt mixtures (0.25 + 0.50), (0.76 + 1), and (0.92 + 1) compared with the experimental water activity measurements for each of them, give a percentage of the recovery that fell in the 95%–105% range for each mixture (Table 4). Thus there was a percentage of recovery of 96%, 103%, and 100% for the mixtures (0.25 + 0.50), (0.76 + 1), and (0.92 + 1), respectively (Table 4).

Supporting these results, water activity measurements compared with the true values for all the standard saturated salt also showed a percentage of recovery within the 95%–105% range. These results demonstrate the reading accuracy of the DPCMM.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for the dew point instrument were determined. The LOD and LOQ were calculated using the standard deviation of the six replicate data obtained for the fewest amount of measurable water activity (aw = 0.25) and the slope of the corresponding standard curve. The LOQ and LOD for the DPCMM were aw = 0.25 and aw = 0.17, respectively. The expected water activity for tablets and capsules

ranges from 0.25 to 0.50, the dew point chilled method thus proving to be a suitable tool for successfully quantifying tiny free water levels while yielding accurate and precise outcomes.

Precision, robustness, and ruggedness

To gain insight into the dew point instrument validation, the repeatability and reproducibility were estimated. The precision of the DPCMM is the degree of agreement among individual test results when the experimental design is applied repeatedly to several samples across the range of the test (six replicates for each solid sample). For this experiment, ruggedness was interpreted as intermediate precision, a type of intra-laboratory precision involving the effect of different batches and operators on the test result variability, as well as the repeatability. To observe the effect of these operational variables on the average water activity, a standard deviation was calculated, and a multifactorial analysis of variance was performed (ANOVA).

As depicted in Table 5, the uncertainties observed by the six replicated from the same batch for the naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol exhibited a standard deviation below 0.01, showing the high concordance precision of the DPCMM values (Table 5). However, for all the products tested, such as naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol, statistical differences were observed among batches (ANOVA, $P < .05$, Table 5). These statistical differences correspond to manufacturing process changes that directly impact the water activity status of the tablets and capsules tested. In spite of the average water activity differences observed among batches, the water activity values exhibited a high degree of concordance (SD < 0.03, Table 5).

It also can be seen that for the DPCMM, the number of different operators does not have a significant effect on the mean of the water activity (Table 6, ANOVA, $P > .05$).

The robustness parameter was assessed following USP guidelines. According to information in chapter 1225, the water activity levels measured by Aqualab 4TE equipment demonstrated its sensitivity to being perturbed by small but deliberate variations in method parameters such as variation in reading temperature (25°C vs. 30°C). In this way, the naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol samples tested showed a percentage of recovery of 98.3114%, 101.3647%, 98.3144%, 101.1330%, 98.3144%, 98.3144%, and 103.1920%, respectively (Table 7). These percentages of recovery are within the specified 95%–105% range, and these differences are statistically different.

Table 4. Percentage of recovery. The mole fraction for each mixture was calculated and compared with the equipment' measured value. Percentage of recovery measured by the dew point instrument should be within the 95%–105% range of the expected true value.

Aqualab 4TE measurement standard 0.25 + 0.50	Mole fraction: 0.25 × 4 ml + 0.50 × 4 ml/8 ml	% Recovery
0.3600	0.3750	96.0000
0.3603	0.3750	96.0800
0.3605	0.3750	96.1300
0.3600	0.3750	96.0000
0.3601	0.3750	96.0300
0.3603	0.3750	96.0800
Aqualab 4TE measurement standard 0.76 + 1.0	**Mole fraction: 0.76 × 4ml + 1.0 × 4 ml/8 ml**	**% Recovery**
0.9087	0.8800	103.2600
0.9076	0.8800	103.1300
0.9076	0.8800	103.1300
0.9077	0.8800	103.1400
0.9082	0.8800	103.2000
0.9083	0.8800	103.2100
Aqualab 4TE measurement standard 0.92 + 1.0	**Mole fraction: 0.92 × 4 ml + 1.0 × 4 ml/8 ml**	**% Recovery**
0.9689	0.9600	100.9200
0.9686	0.9600	100.8900
0.9684	0.9600	100.8700
0.9691	0.9600	100.9400
0.9694	0.9600	100.9700
0.9686	0.9600	100.8900

Microbiological test

For each sample of naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol tested which has water activity under specification (aw < 0.60), the microbiological test results for mesophiles (counts < 10 cfu/g), *E. coli* (absent), yeast and molds (counts < 10 cfu/g) fit microbiological specification. Therefore, water activity status could be considered as a

Table 5. Ruggedness testing: water activity average obtained by different batches. Water activity measured at 25°C for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol. For each sample, six replicates were taken to calculate mean and SD.

	Lot 1		Lot 2		Lot 3		ANOVA *P* values
	Mean $n = 6$	SD	Mean $n = 6$	SD	Mean $n = 6$	SD	
Naproxen	0.4300	0.0013	0.4267	0.0014	0.4250	0.0013	0.0000
Amoxicillin	0.4759	0.0007	0.5088	0.0005	0.4462	0.0003	0.0000
Ciprofloxacin	0.4545	0.0012	0.4485	0.0019	0.3424	0.0017	0.0000
Chlorpheniramine	0.4570	0.0013	0.4419	0.0021	0.4246	0.0106	0.0000
Prednisolone	0.4015	0.0007	0.4055	0.0013	0.4075	0.0002	0.0000
Flunarizine	0.3715	0.0023	0.3358	0.0029	0.4718	0.0006	0.0000
Methocarbamol	0.4850	0.0008	0.5091	0.0038	0.5068	0.0036	0.0000

Table 6. Ruggedness testing: water activity average obtained by different operators for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol.

Product name	Operator 1 $n = 18$	Operator 2 $n = 18$	ANOVA P value
Naproxen	0.4272	0.4265	0.317
Amoxicillin	0.477	0.4715	0.508
Ciprofloxacin	0.4151	0.4128	0.985
Chlorpheniramine	0.4412	0.4322	0.115
Prednisolone	0.4048	0.4049	0.941
Flunarizine	0.393	0.3892	0.851
Methocarbamol	0.5003	0.5003	0.991

Table 7. Robustness testing: water activity average obtained at different temperatures (25°C vs. 30°C) for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol.

Tablets and capsules	25°C Mean $n = 6$	30°C Mean $n = 6$	Percentage of recovery
Naproxen	0.4300	0.4090	98.3144
Amoxicillin	0.5088	0.5019	101.3647
Ciprofloxacin	0.4545	0.4352	98.3144
Chlorpheniramine	0.4419	0.4369	101.1330
Prednisolone	0.4015	0.4084	98.3144
Flunarizine	0.3715	0.3917	98.3144
Methocarbamol	0.4850	0.4702	103.1920

reliable measure for microbiological burden at least in the solid sample tested. Moreover, the solid samples tested have fulfillments of microbiological historical results of at least the last 20 batches of the finished product, raw material, and primary packaging.

Conclusions

In this study, carried out in Coaspharma laboratories, the suitability of the method for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol was demonstrated. Normally, tablets and capsules take around 1 min to be taken out of the blister pack as the units are placed into the plastic cup at the same time as the cup is placed into the measuring chamber as soon as possible. This information is remarkable because it ensures that the previous step before the sample reaches the instrument neither absorbs nor loses water activity, yielding uncertainties in water activity measurements. As has been shown for the suitability of the method, sample preparation and exposure times of 5 min exhibit water activity differences that fall in the expected 95%–105% range. However, the water activity averages were statistically different (ANOVA, $P < .05$).

Despite statistical differences in the sample preparation and exposure times for all the samples tested, these differences fall into the percentage recovery 95%–105% range. It is noteworthy to consider that the average water activity for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol is around 0.50, so differences of more than 5% respect to the overall free water activity never will be more than to the specified value for water activity (aw = 0.60), ensuring a precise microbiological assessment.

Furthermore, it was also demonstrated that based on calibration curves using saturated salts, it was possible to establish the linearity and operating range of the DPCMM. The evidence demonstrates that this alternative automated method yields precise and accurate results ($R^2 = 1$, CC = 1, % recovery >99%). 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 $P < .05$), those differences corresponded to manufacturing process variations that impacted the water activity status of naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol. Moreover, the DPCMM shows a high degree of concordance (SD < 0.01).

As can be observed in the calibration curves, the limits of detection and limits of quantification were 0.17 and 0.25, respectively. These results demonstrate the good performance of the dew point instrument in detecting the lowest water activity, ensuring an accurate assessment of the water activity status of the solid samples tested. For instance, the average water activity for naproxen, amoxicillin, ciprofloxacin, chlorpheniramine, prednisolone, flunarizine, and methocarbamol was 0.4272, 0.4770, 0.4151, 0.4412, 0.4048, 0.3930, and 0.5003, respectively. 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].

All these water activity calculated for the solid pharmaceutical matrix 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 *B. cepacia* complex and *E coli* in tablets and capsules with water activity lower than 0.60.

Nonetheless, as has previously been shown, the uncertainty of water activity measurements in the pharmaceutical matrix with a high concentration of non-aqueous materials such as ethanol and propylene glycol may strongly impact the accuracy of the DPCMM [20]. This is a notable issue that has to be considered when a sample to be validated is chosen to avoid uncertain results. However, considering the negligible amount of volatiles other than water, such as ethanol and propylene glycol, in the tablets and capsules tested, the DPCMM gives precise and accurate results.

The use of such alternative methodologies results in a reduction of company warehousing costs, improved efficiency in inventory control, and the ability to respond more quickly to adverse microbiological results, as well as leading to fewer waste products.

Author contributions

P.R., J.J., and J.C. conceived and designed research. P.R. wrote the manuscript. All the authors read and approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

All the data underlying this article are available in the manuscript

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