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Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1352–1357
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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# Development and validation of a high-throughput GC measurement for water activity

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Received 26 September 2006; received in revised form 31 October 2006; accepted 3 November 2006
Available online 12 December 2006

**Abstract**

The development and validation of a headspace GC method to measure the water activity of pharmaceutical samples is presented. Thermal and moisture transfer equilibration rates are shown to be critical variables in the measurement. Several different calibration schemes are discussed with their advantages and disadvantages. The high-throughput applications and experimental considerations of this approach are discussed. The method is shown to be a useful tool to measure a high throughput of water activity samples.


*Keywords:* GC; Water activity; Microbial limits

## 1. Introduction

Moisture is an important variable effecting pharmaceutical quality. At high moisture levels, degradation rates are often faster [1], formulation release characteristics change [2–4], and microbial propagation can increase to unacceptable levels [5]. Yet, all water is not equal. Water bound up in crystalline hydrates is not accessible in most cases to participate in chemical reactions or to facilitate microbial propagation. Different formulations likewise, vary significantly in their sensitivities to moisture. A strongly hydrophilic formulation may have greater overall moisture content than a hydrophobic formulation and still have much less chemical impact from that moisture. Thus, measurements of the total water of a system must be placed in the context of the moisture sorption properties of the matrix.

A more general and chemically relevant measure of the overall impact of the moisture of a formulation is found in its water activity ($a_w$). Water activity is the measurable manifestation of the steady state chemical potential of water within a sample through its effects on its surroundings and can be thought of as the gas phase relative humidity (%RH) at equilibrium with a condensed phase sample. Water activity values are often presented as a unitless decimal from 0.0 to 1.0, equivalent to %RH/100. Because water activity is a measurement of the small portion of a sample's moisture content that partitions into the gas phase, it allows measurement of the overall water content of a sample without the need to quantitatively extract the moisture from the sample. Only a very small relative proportion of the overall sample moisture is volatilized into (or absorbed from) the gas phase when a sample achieves thermodynamic equilibrium with its surroundings. Therefore, the measurement of the equilibrium percent relative humidity of the air surrounding a sample provides a non-destructive moisture analysis that allows subsequent use of the water activity samples for other analyses. Further, equilibrium can be established without the need for grinding of the dosage form, greatly reducing the potential for environmental biases on the moisture measurement.

The water activity of a sample is a variable of both the total water in the sample as well as its moisture absorbing properties. Water activity provides a useful measure of the chemical accessibility of the water content of a sample and provides a relevant frame of reference to monitor the equilibration of a sample to its surrounding relative humidity environment (*e.g.*, ingress of moisture into packaged drug product). Water activity

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doi:10.1016/j.jpba.2006.11.016

is a much more accurate predictor of pharmaceutical degradation rates [1] and microbial growth [5] than total water content, and is directly comparable between samples of different moisture sorption properties. Because water activity is a strong predictor of microbial growth, it has been suggested as surrogate for compendial microbial limits testing for samples dry enough to prevent microbial propagation [6,7]. In this role, it provides a powerful triage of microbial testing samples, to focus attention and resources only on those samples capable of supporting undesirable microbial activity.

Numerous different water activity measurement methods have been in wide use in the food science community for many years, including gravimetric, psychrometric, hygrometric, and direct manometric approaches [8]. The use of water activity measurements in the pharmaceutical industry is more recent and has relied primarily on off-the-shelf analytical instruments. The most popular techniques for water activity measurement in the pharmaceutical industry are the chilled mirror method [6,7,9], frequency modulated spectroscopy [10], electric hygrometry, and headspace gas chromatography [11]. Of these methods, headspace GC holds the most significant potential for convenient automation of the water activity measurement with off-the-shelf analytical hardware.

To be meaningful, water activity requires the context of temperature. As temperature increases, more of a sample's water content is partitioned into the gas phase, but the saturation vapor pressure of water likewise increases with temperature. Both of these increase with temperature to differing degrees, leading to a product-specific temperature dependence of the water activity measurement. The net result of this balance is that the water activity of tablet samples of constant moisture content often varies by 10%RH or more across a measurement temperature range of 25–40 °C, and the water activity measurement has meaning only when presented together with the measurement temperature. Well-controlled thermal equilibrium of the sample is thus as important to the water activity measurement as the quantitation of vapor phase water itself. Typical temperatures of pharmaceutical interest span the ICH stability conditions from 25 to 40 °C. The current work discusses the successful application of this headspace GC analytical approach to the measurement of headspace water activity under these pharmaceutically relevant conditions.

## 2. Experimental

All gas chromatography experiments were conducted with an Agilent 6890 gas chromatograph interfaced with a Leap Technologies CombiPAL headspace autosampler. Many GC autosamplers cannot maintain good temperature control in the 25–40 °C temperature range, and we have specifically selected the Leap Technologies autosampler in this study for its abilities to control sample temperature within a ±1 °C tolerance within this temperature range. Chromatographic separation was achieved by using a 60 m × 0.53 mm Restek Stabilwax column with a film thickness of 1.5 μm. Detection was by thermoconductivity. Table 1 lists the important separation and autosampler parameters.

Table 1
Instrumentation and conditions

| Leap Technology autosampler configurations | |
|---|---|
| Incubation temperature (°C) | 25, 30, and 40 |
| Incubation time (min) | 60 |
| Injection volume (ml) | 0.250 |
| Syringe volume | 1 ml headspace syringe |
| Syringe temperature (°C) | 30, 35, and 45, respectively |
| Agitator speed (rpm) | 250 |
| Fill speed (ml/s) | 0.100 |
| Fill strokes | 0 |
| Pull-up delay (ms) | 0 |
| Injection speed (ml/s) | 1.000 |
| Pre inject delay (ms) | 0 |
| Post inject delay (ms) | 0 |
| Flush time (s) | 360 |
| GC run time (s) | 400 |
| Agilent 6890 GC configurations | |
| Carrier gas | Helium |
| Inlet temperature (°C) | 150 |
| Initial flow (ml/min) | 8.0 |
| Flow mode | Constant flow |
| TCD temperature (°C) | 240 |
| Makeup gas (ml/min) | 5.0 |
| Reference flow (ml/min) | 40 |
| Oven program | 125 °C isothermal |
| Run time (min) | 5 |

A Lighthouse Instruments FMS 1400 Headspace Moisture analyzer (Lighthouse Instruments, Charlottesville, VA) was used for frequency modulation results presented in this work. The optical head where the sample container is presented (accessible from the top) is continuously purged with nitrogen to eliminate any room air moisture from the measurement region prior to conducting experiments. Containers are inserted to the sample holder from the top of the instrument and the measured moisture concentration is sent to the computer as well as the instrument front panel display. The sample holder is jacketed and the temperature of the sample holder can be controlled.

Typical operation of the instrument was conducted in the following sequence: the sample holder was heated/cooled to the desired measurement temperature. Next, the instrument was calibrated at the measurement temperature by using a known activity solution. The water activity reading was graphically monitored in real time to ensure that the vial headspace had indeed reached equilibrium with the sample at the measurement temperature. After the instrument was calibrated, a similar procedure was followed for the actual measurement. Each time the temperature or container was changed or the instrument was shut down, a new calibration was performed.

## 3. Sample/standard preparation

Saturated salt solutions were used as standards for the water activity measurement [12]. The theoretical %RH values for the most common saturated salt solution standards are given in Table 2 and come from Ref. [12]. In addition to saturated salt solutions, anhydrous magnesium perchlorate (a drying agent)

Table 2
Salt solutions for maintaining constant humidity

| Saturated salt solution | %RH at specified temperatures (°C) | | |
|---|---|---|---|
| | 25 | 30 | 40 |
| $LiCl{\cdot}H_2O$ | 10.2 | 12.0 | 11.0 |
| $MgCl_2{\cdot}6H_2O$ | 33.0 | 33.0 | 32.0 |
| $K_2CO_3{\cdot}2H_2O$ | 42.8 | NR | 42.0 |
| $Mg(NO_3)_2{\cdot}6H_2O$ | 52.9 | 52.0 | 49.0 |
| $Na_2Cr_2O_7{\cdot}2H_2O$ | 54.0 | NR | 53.6 |
| $NaBr{\cdot}2H_2O$ | 57.7 | NR | 52.4 |
| $NaNO_3$ | 73.8 | 72.8 | 71.5 |
| NaCl | 75.3 | 74.9 | 74.7 |
| KCl | 84.3 | 84.0 | 81.7 |

NR: not reported in Ref. [12].

was used to determine the residual moisture of instrument as well as to demonstrate specificity of the measurement.

Standards were prepared by adding 2 ml of water to about 10 g of salt in a 20 ml headspace GC vial (MicroLiter Analytical Supplies Inc.). The vials were then sealed (airtight) and left at room temperature for 48 h to achieve full moisture equilibration. Three replicate vials were prepared for each salt solution. Vials were then analyzed using the GC method described above. To remeasure vials, the vials were vented to atmospheric pressure and allowed to re-equilibrate over 24 h. The analysis of water was performed at 25, 30 and 40 °C. External standard quantitation is performed using one or several saturated salt solutions – typically NaCl (aq) and/or LiCl (aq) – using typical chromatographic procedures. Method precision samples were preconditioned in an environment of known water activity using stability chambers or saturated salt solutions prior to analysis, and the temperature setting on the Leap autosampler was fixed at the preconditioning temperature during the measurement.

## 4. Results and discussion

### 4.1. Specificity

As shown in Fig. 1, the headspace GC method produces excellent resolution of water from all interfering peaks in 5 min

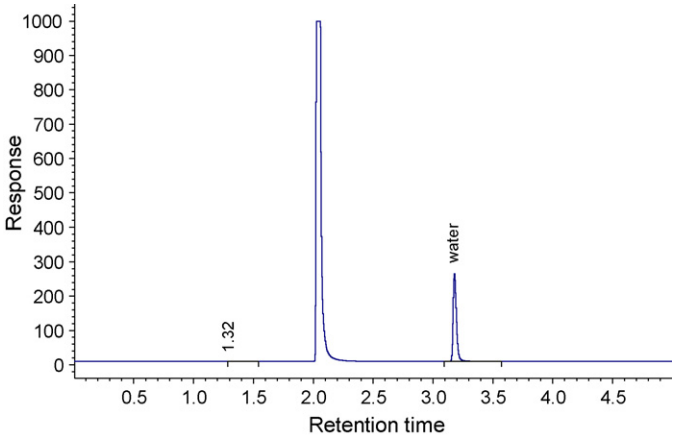


Fig. 1. Representative chromatogram for headspace water activity of a tablet sample.

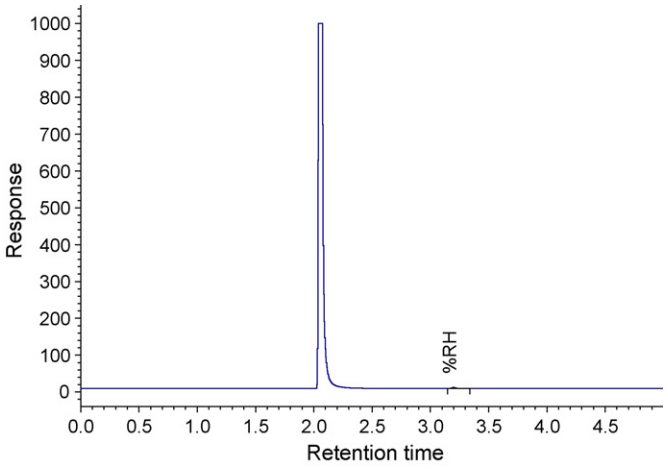


Fig. 2. Representative chromatogram for water vapor carryover in a "dry" sample containing $MgClO_4$ drying agent.

analysis time. Water is retained 1 min from the unretained air peak. The water peak is slightly tailing (tailing factor = 1.25) but acceptable for accurate quantitation. Water is a difficult analyte in general, and this degree of tailing is not surprising. This headspace GC analysis is unusual in that the analyte (water) is present as well at analytically significant levels in the ambient air, and systematic biases due to carryover from ambient moisture is a potential method issue. Indeed, the injector needle is exposed to the ambient air in between injections. However, the impact of carryover is dramatically reduced with a continuous helium flush of the syringe needle between injections. Water may be absorbed to the syringe surface at the beginning of the experiment, and the first several injections in a run are often systematically high unless a helium flush of the injector needle is performed. In practice, the lowest measurable water activity measurable was ~2–3%RH at 25 °C and 0.9%RH at 40 °C, even in the presence of magnesium perchlorate drying agent (Fig. 2). It should be noted that the absolute water vapor pressures responsible for the 40 °C bias is approximately equivalent to that of the 25 °C bias, and differs largely in the equilibrium vapor pressure of water that it is compared to. This observation in itself is highly suggestive of a basic level of carryover from the ambient environment. Memory effects between injections is not observed, even for the measurement of very dry samples after very wet samples and *visa versa*.

### 4.2. Moisture equilibration

The time required to achieve full moisture equilibrium was examined for a number of different types of dosage forms and saturated salt standards by measuring water activity as a function of equilibration time. The equilibration is quite rapid for the saturated salt solutions due both to good thermal contact with the vial wall, and to the rapid mass transfer of the solution state. Non-film coated tablets and hard gelatin capsules achieved a steady state of equilibration in ~30 min, and film coated tablets and softgel capsules took as much as 2 h to achieve steady state water activity values. In the former case, much of this slower equilibration can be attributed to slower thermal equilibration of the vial contents

with the vial surface temperature, but in the latter cases, slowed moisture equilibration in the system is almost certainly the dominant factor. Conservative equilibration times of 1 or 3 h were used for these two types of samples in all measurements reported below. In practice, these long equilibration times do not significantly slow the measurement due to the ability to equilibrate more than one sample at once. The heater/agitator of the Leap CombiPAL autosampler is capable of equilibrating six samples simultaneously at, reducing the duty cycle to only 10 and 30 min, respectively, for 1 and 3 h sample equilibration times. Alternatively, samples can be thermally equilibrated directly on the sample tray, which has temperature control capabilities from 4 to 70 °C. This latter approach provides a desirable efficiency advantage, reducing the duty cycle to the chromatography length of 5 min per sample. However, this is only appropriate in practice for 25 and 30 °C samples due to a tendency for condensation to develop at the top of vials containing high moisture samples at 40 °C. This condensation represents a non-equilibrium two-phase system and results in erroneously high water activity being measured for these samples. The origin of this condensation can be understood in the context of the quality of thermal equilibration of the overall GC vial. The bottom of the vial where the sample is located is encased within the aluminum heating block and is heated reproducibly to the desired 40 °C. However, the top of the vial projects from the heating block and is cooled somewhat by ambient air, resulting in a cooler surface for condensation to appear on. Measurement of the vial tops for vials heated in the sample tray to 40 °C reveals that these vial tops can be as low as 27 °C in a controlled room temperature lab environment of ~20 °C. Since the saturation vapor pressure of water doubles between 25 and 40 °C, samples with a true 40 °C water activity of as little as 55%RH may experience condensation at the vial tops. It is thus recommended that all 40 °C measurements be conducted with thermal equilibration in the more thermally contained heater/agitator and that 25 and 30 °C measurements be conducted with thermal equilibration in the sample tray. All results reported below were conducted using these optimal practices.

### 4.3. Linearity/accuracy

The linearity and accuracy of the GC water activity method was assessed by quantitating the water activity of a series of well-characterized saturated salt solution standards and correlating the measured versus theoretical values. Because the method was known to possess a slight positive bias at low water activities, solutions were quantitated against both a single saturated NaCl standard as well as a two-point calibration anchored by LiCl and NaCl saturated solutions. In all cases (25, 30, 40 °C measurements with one- and two-point calibration), the linearity of the best-fit line is good, with $R^2 > 0.996$. However, the linearity and accuracy at 25 and 30 °C was noticeably better than at 40 °C, perhaps due to the more complicated analysis potential for biases arising from condensation at 40 °C (vide supra). All 25 and 30 °C analyses had $R^2 > 0.999$. The accuracy for 25 and 30 °C analyses was excellent, with all measured values ±2%RH of theoretical with the two-point calibration (Fig. 3). When quantitated against a single saturated NaCl standard, the accuracy at high water activity was generally similar to that measured with a two-point calibration, and decreased gradually at low water activity to a maximum error of 4%RH for LiCl solutions (theoretical %RH = 10.2) (Fig. 4). Measurements at 40 °C had errors as high as 5%RH, and in almost all cases, the errors were systematically higher than the theoretical values, leading to a slightly greater than unity slope to the best-fit line. This relationship between measured and theoretical water activity values strongly suggests the presence of a systematic bias to the analysis. It is probable, that this bias results from the temperature gradient in the system, and reflects a non-equilibrium state at the time of analysis.

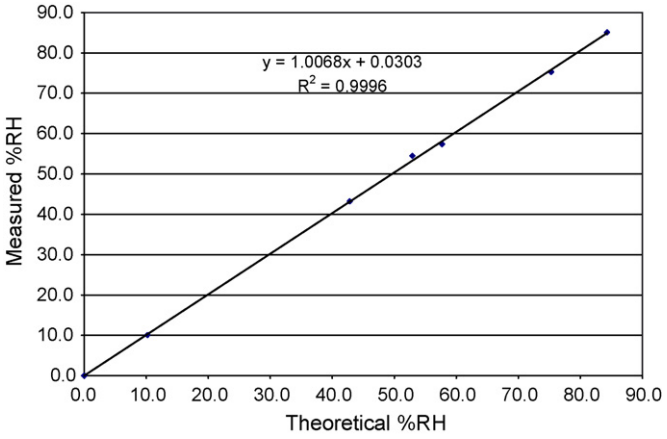


Fig. 3. Measured vs. theoretical water activity measured at 25 °C vs. two-point calibration anchored by saturated NaCl and LiCl solution standards.

### 4.4. Precision

The precision of the analysis between nominally equivalent saturated salt solution standards was quite good, with seven injections of saturated NaCl solutions ranging between 0.9 and 1.3% R.S.D. for 25, 30, and 40 °C measurements. The precision of other salt solutions was generally related to the equilibrium %RH, with higher RH samples giving slightly lower % R.S.D.s

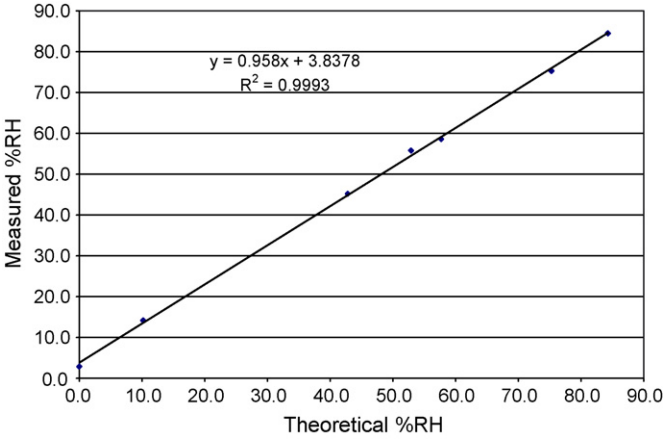


Fig. 4. Measured vs. theoretical water activity measured at 25 °C vs. a saturated NaCl solution standard.

due to higher overall analytical signal and lesser chance of environmental biases.

### 4.5. Limit of quantitation

Both experiments in the presence of drying agent and the least squares fitting of the linearity of saturated salt solution standards suggest the presence of a positive systematic bias of ∼3% for measurement at very low %RH measured relative to saturated sodium chloride solutions as a standard. This bias diminishes significantly as the measured RH rises and becomes similar to or greater than the ambient RH. The LOQ under this quantitation mode is estimated at ∼5% due to this significant systematic error in low water activity measurements. Sample quantitation relative to a two point standard curve anchored by saturated LiCl solutions on the low end and NaCl solutions on the high end effectively removes this bias and allows measurement of near-zero water activities. When this two point calibration approach was used, routine measurement of samples <1%RH was possible, though at a slight sacrifice of quantitation accuracy at high %RH. To the extent that water activity is used to identify samples with high enough water activity to be at risk for microbial growth (*i.e.*, >60%RH), this bias at the low end of the scale is acceptable. However, for the measurement of dry samples, the two-point calibration approach may significantly improve accuracy and allow an LOQ of ≤1%.

### 4.6. Comparison to FMS

The equivalence of the GC method was evaluated relative to a frequency modulated spectroscopy (FMS) method that has been reported previously [10]. This method is slower and non-automated, but has several advantages in terms of sample handling in that the sample remains sealed in a moisture impermeable glass vial prior to and during the analysis. Comparative GC and FMS measurements were conducted on a wide variety of developmental pharmaceutical formulations with a large range of actual water activity levels spanning the range of interest. The collective results are shown in Fig. 5 below. In general, the results agree within 4%RH, which is an acceptable degree of agreement, given the assumed variability of ±2%RH for each measurement and the potential for small environmental biases between the two tests due to the time lag between measurements and the necessary sample handling to repackage FMS samples in GC vials for reanalysis.

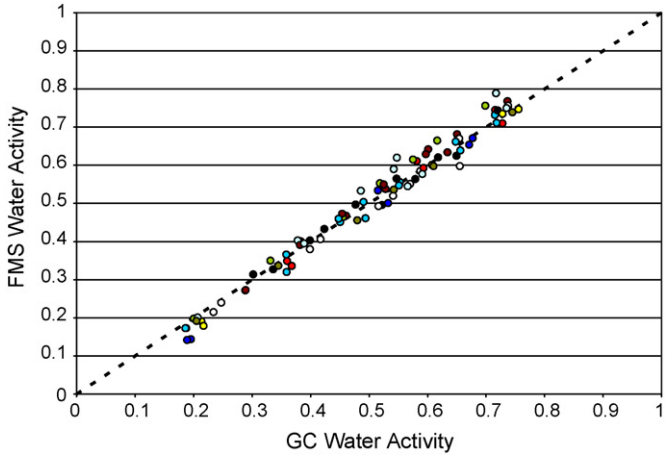


Fig. 5. Comparison of headspace GC and frequency modulated spectroscopy (FMS) results for 88 pharmaceutical tablet samples spanning the water activity range of interest. The dotted line represents the theoretical slope of 1.0. Samples were measured at 25, 30, or 40 °C, and GC and FMS analysis temperatures match in all cases.

## 5. Conclusions

In the discussion above, we have demonstrated the feasibility of using automated headspace GC to provide water activity measurements with high throughput. This approach is quite general and is implementable with a wide array of commercial GC instruments. The main caveat for this is the control of temperature. Many commercial GC autosamplers have minimum effective temperatures that are above the 25–40 °C range most relevant to water activity measurements. In this work, we have configured one practical off-the-shelf instrumental setup that meets the needs of near-ambient temperature water activity measurement. However, many other different, but equally satisfactory configurations may be imagined as long as explicit consideration is given to appropriate thermal equilibrium. The true efficiency of the method is achieved at 25–30 °C, where samples can be left to equilibrate under near-ambient conditions on the GC tray and injected as fast as the GC cycle time will allow without further consideration of thermal or moisture equilibration. In practice, we see that this leads as well to optimal performance of the analytical method by eliminating biases due to incomplete equilibration or condensation. Under the relatively moderate analysis conditions described in this work, 60 or more water activity samples can be analyzed in an 8 h shift with minimal analyst intervention.

## Acknowledgments

The authors gratefully acknowledge the contributions of Richard Eddy of Eddy Analytical Solutions in optimizing the instrumental configuration to achieve optimal accuracy and robustness. The authors would also like to thank Justin Brennan and David Hall for help in collection of some of this data and Dr. Rey T. Chern for useful discussions.

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