

Short communication

Evaluation of methods for the determination of water in substances with unknown chemical and thermal behaviour

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Received 22 June 2006; received in revised form 2 August 2006; accepted 2 August 2006

Available online 7 September 2006

Abstract

The water content of six different substances was determined by means of five different methods. The methods used were loss on drying, thermogravimetry (coupled with DSC), a new phosphorous-pentoxide method, direct Karl–Fischer titration and the Karl–Fischer oven. A combination of direct Karl–Fischer titration and the KF-oven method was found to be the optimal technique for water determination of substances of unidentified chemical and thermal behaviour based on detailed considerations about selectivity, type of water binding, sample properties and efficiency. © 2006 Elsevier B.V. All rights reserved.

Keywords: Water content; Thermal analysis; Loss on drying; Thermogravimetry; Karl–Fischer titration; Phosphorous pentoxide sensor; Method development

1. Introduction

The water content, along with the residual solvent content and the content of inorganic components (sulphated ash), represents a base parameter, which has to be determined frequently in the pharmaceutical industry [1]. Quality control during the production of active ingredients requires an uncomplicated and possibly automatable method in order to attain the required efficiency and to master the number of samples. This is why loss on drying is popular for water determination, possibly even for determining the total amounts of water and residual solvents. Since the substance behaviour concerning sublimation or side reactions is already known during the production process, the use of such non-specific methods is possible and understandable. During the development of an active-ingredient synthesis, however, it is necessary to use a method that delivers correct and fast results even when examining the various substance classes of starting materials, intermediate products all the way up to the active ingredient. Thus, non-specific and time-consuming methods cannot be used. For these reasons, the water content of various substance classes should be determined by using diverse methods in order to judge the significance of the water

determination methods and to evaluate, if possible, a standard method, which can be used when little is known about substance behaviour.

Loss on drying is one of the simplest water determination methods. The loss on drying is the mass loss indicated in percent, which is attained by drying at a given temperature and pressure. It is dried until the uniformity of mass has been achieved [2].

A combination of thermal analysis and coulometry is used in the new phosphorous-pentoxide method, which Sartorius AG (Göttingen, Germany) developed [3]. The sample is introduced into an oven by using a sample scoop and the water is thermally driven out of the sample material in the nitrogen stream. The water detection is accomplished by an electro-chemical sensor (phosphorous pentoxide between two electrodes arranged in parallel) allocated to the coulometric measurement procedure.

Thermogravimetry is an analysis procedure, with which the change in weight of a sample is measured during a given temperature-time-program in a defined gas atmosphere. This is usually coupled with other methods, such as differential scanning calorimetry (DSC) [4], in order to be able to assess the temperature-dependent processes that occur, such as vaporisation, sublimation or decomposition.

The most important chemical procedure for water determination is the one named after its discoverer, i.e. the Karl–Fischer method [5]. The procedure is based on the principle that iodine and sulphur dioxide react with one another only in the presence

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of water. The protons have to be intercepted in order to effect this quantitative balance reaction. This was achieved by adding pyridine, which nowadays has been replaced by imidazole [6]. Methanol or diethylene glycol monoethyl ether serve as solvents, which dissolve all components, stabilise the titration solution and also takes part in the reaction, as further examinations of the reaction mechanism by Smith et al. [7] have shown.

With the KF-oven method, the substance to be examined is weighed directly into the sample vials, which is heated to a maximum of 250 °C in an oven. A dry carrier gas stream is pumped via the inlet needle through the heated sample. The released moisture flows directly into a titration cell where the determination is carried out using volumetric or coulometric Karl–Fischer titration [8].

There is a multiplicity of other methods for the determination of water, such as near-infrared spectroscopy, toluene distillation or gas chromatography, to which, however, no further attention is going to be paid during the course of this study, since they are too time consuming and/or require complex calibration or large quantities of samples.

2. Experimental

2.1. Materials

Six different substances were selected for comparison, which have been classified as critical with regard to the determination of water in routine daily work because they have a low boiling point or tightly bound water of crystallisation, cause possible side reactions and are difficult to handle: piperazine hexahydrate (98%, Acros Organics, Geel, Belgium), triphenylphosphine (Atofina Deutschland GmbH, Düsseldorf, Germany), magnesium sulphate heptahydrate (p. A., Merck, Darmstadt, Germany), Kromasil (NP 10 µm/100 A, EKA Chemicals AB, Bohus, Sweden), Hydranal-Water Standard KF Oven (water content: $5.55 \pm 0.2\%$, Riedel-de Haen, Seelze, Germany), and one of our own active pharmaceutical ingredients (a fluorine-nucleoside derivative), which will be abbreviated as API in the following.

2.2. Equipment and conditions

The water content of these substances was comparatively analyzed by means of classical loss on drying, phosphorous-pentoxide method, thermogravimetry (in combination with DSC), Karl–Fischer titration and the Karl–Fischer oven method. For the determination of loss on drying, approximately 1 g substance was dried for 3 h at 105 °C in a drying cabinet for each sample and the resulting loss of mass was determined by the difference in weight. For the P₂O₅ method the apparatus, WDS 400, manufactured by Sartorius (Göttingen, Germany) was used. For the determination process and depending upon water content, 2–100 mg sample were weighed onto aluminium sample scoops and then placed into the oven tube. Depending upon the water type, different temperature programs were chosen for the determination of water, i.e. for piperazine hexahydrate [36 °C (4 min), 14 °C/min, 50 °C (7 min), 90 °C/min, 90 °C

(9 min)], triphenylphosphine [36 °C (2 min), 38 °C/min, 150 °C (5 min), 17 °C/min, 200 °C (4 min)], magnesium sulphate heptahydrate [105 °C (5 min), 59 °C/min, 400 °C (35 min)], Kromasil [40 °C (2 min), 22 °C/min, 105 °C (5 min), 12 °C/min, 200 °C (22 min)] and Hydranal-Water Standard KF Oven [40 °C (2 min), 22 °C/min, 105 °C (5 min), 12 °C/min, 200 °C (5 min), 17 °C/min, 250 °C (14 min)]. The Karl–Fischer oven method was carried out with a combination of Oven Sample Processor 774 and diaphragm-less Coulometer 831, manufactured by Metrohm (Herisau, Switzerland). Hydranal Coulomat AG Oven (Riedel-de Haen, Seelze, Germany) served as reagent. For determination, approximately 30 mg of each sample were weighed into glass vials and then analyzed. After recording a warming curve, the extraction temperature was selected, i.e. for triphenylphosphine, 50 °C; magnesium sulphate heptahydrate, 220 °C; Kromasil, 150 °C; Hydranal-Water Standard KF Oven, 220 °C, and for the API, 150 °C. A system made up of 702 SM Titrino and 703 Ti Stand, manufactured by Metrohm (Herisau, Switzerland) was used for direct Karl–Fischer titration. Hydranal Solvent and Hydranal Titrant 5 (Riedel-de Haen, Seelze, Germany) served as reagents, whereby, depending upon water content, samples of between 20 and 100 mg were used.

The apparatus, TGA850 (Mettler Toledo, Giessen, Germany) was used for the thermogravimetric examination, which is combined with DSC and therefore permits information on exothermic and endothermic reactions. Analysis was conducted in four-fold determination with each 5–10 mg of substance in aluminium-crucibles (100 µL volume) under nitrogen (50 mL/min) with a temperature program (25 °C, 5 °C/min, 400 °C). A survey analysis was conducted during the preliminary stages with a temperature program from 25 to 1000 °C set at 10 °C/min.

3. Results and discussion

3.1. Piperazine hexahydrate

The results of each four-fold determination are compared in Table 1. Piperazine hexahydrate is a substance that has a low melting point and a low boiling point (mp: 44–45 °C, bp: 145–156 °C [9]). The application of thermal procedures, therefore, results in incorrect results, or respectively, in the indeterminability of the water content. Only direct Karl–Fischer titration is successful in determining the correct water content, which is mainly caused by the water of crystallisation. No determination can be conducted with the WDS P₂O₅ method and the KF-oven method because the substance contaminates the P₂O₅ sensor and/or the transfer tubes. Due to the coated P₂O₅ sensor at the WDS P₂O₅ apparatus, the absence of water was falsely indicated because the sensor failed to show any malfunction (as a self test). Also, the relatively low temperatures of 105 °C, attained during the loss-on-drying method, result in values that are too high, which can be attributed to a reduction in substances that become volatile even at these temperatures. Also thermogravimetry shows from the beginning an overlay of dehydration by the boiling of the substance.

Table 1
Water contents ($n=4$) of the six substances according to the five test methods

	Loss on drying		P ₂ O ₅ method		Thermogravimetry		Karl–Fischer titration		KF-oven method	
	Mean (%)	Standard deviation (%)	Mean (%)	Standard deviation (%)	Mean (%)	Standard deviation (%)	Mean (%)	Standard deviation (%)	Mean (%)	Standard deviation (%)
Piperazine hexahydrate	83.51	1.25	–	–	–	–	55.12	0.16	–	–
Triphenylphosphine	0.15	0.04	0.01	0.00	0.00	0.00	6.84	0.19	0.01	0.01
Magnesium sulphate heptahydrate	35.97	0.07	52.11	4.72	52.45	0.16	53.71	1.47	47.79	0.56
Kromasil	1.08	0.01	0.64	0.07	0.98	0.07	0.52	0.08	0.45	0.01
API	–	–	–	–	5.26	0.23	5.80	0.15	5.56	0.19
Hydranal KF-Oven Standard	0.03	0.01	5.60	0.07	5.74	0.11	5.81	0.29	5.50	0.06

3.2. Triphenylphosphine

In the case of triphenylphosphine, there was no dehydration to be seen during thermogravimetry (TG-DSC) until about 170 °C; afterwards, there was a noticeable mass reduction caused by boiling. Both of the oven methods, the P₂O₅ method and the KF-oven method identified a water content of 0.01%. Determination by loss on drying shows an elevated value of 0.15%, which is, nevertheless, not real but attributable to the evaporation of triphenylphosphine, itself (mp: 80 °C, bp: 188 °C [10]). Direct Karl–Fischer titration shows the highest water content at 6.84%, which is due to the fact that the substance, as a reducing agent, reacts with the iodine of the Karl–Fischer reagent and, in doing so, simulates water.

3.3. Magnesium sulphate

The magnesium sulphate is not critical with regard to the vaporisation of the substance. The water of crystallisation is, however, very tightly bound. Thermogravimetry, as well as the P₂O₅ method show results comparable to those obtained with direct Karl–Fischer titration. Loss on drying shows inaccurate readings that are substantially too low due to the low bake-out temperature of 105 °C. Likewise, the KF-oven method shows inaccurately low findings. This is attributable to the fact that the oven used in the KF-oven method was operated at 220 °C, since the release curve (50 °C, 2 °C/min, 250 °C) did not show any further release from about 200 °C. The KF oven of the Metrohm apparatus can, however, be heated generally only to a maximum of 250 °C. The oven of the Sartorius apparatus (P₂O₅ method) can be heated to 400 °C. The bake-out temperature of 220 °C is not sufficient for the water of crystallisation of magnesium sulphate.

3.4. Kromasil

The water contents determined for Kromasil vary depending upon the method of determination. Kromasil was not soluble in the working medium (Hydranal Solvent, Riedel-de Haen, Seelze, Germany; containing imidazole and sulphur dioxide dissolved in methanol) used for direct Karl–Fischer determination, which resulted in inaccurately low findings when compared to

loss on drying and thermogravimetry. Nonetheless, the inaccurately low results of the P₂O₅ method and the KF-oven method are amazing when compared to loss on drying, to which a possible inhomogeneity of the samples may be connected, since the determination of water in Kromasil with all the methods of determination, except for loss on drying, exhibits the highest relative standard deviation (standard deviation/mean). Only with loss on drying 1 g portions were analyzed. The sample quantities for the other determination procedures ranged between 2 and 100 mg, which makes a stronger vulnerability to sample inhomogeneities possible. The probability of inhomogeneity of the sample is confirmed by taking a closer look at the type of water binding. As the temperature-related release of water in the P₂O₅ method has shown (cf. Fig. 1), this was mainly surface water, which is released at temperatures as low as 40 °C.

3.5. Active pharmaceutical ingredient (API)

The determination of water in the active pharmaceutical ingredient (API) was not possible with loss on drying and the P₂O₅ method because of the static charge of this substance. The micronised active pharmaceutical ingredient could not be introduced into the equipment in the weighing containers, since, due to the static charge of the micronised active ingredient, the substance leapt from the containers onto the walls. The substance could only be introduced into the systems in closed containers, as was the case with the other methods.

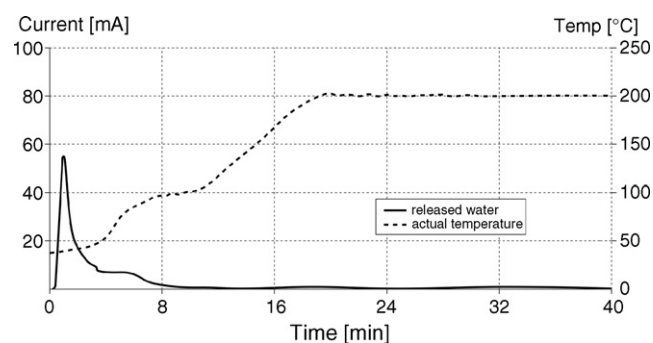


Fig. 1. Progression of the release of water as a function of time or respectively of temperature at a given temperature program [40 °C (2 min), 22 °C/min, 105 °C (5 min), 12 °C/min, 200 °C (22 min)].

Table 2
Evaluation of the water determination methods according to categories

Selectivity	Type of water binding	Sample properties	Efficiency
Loss on drying Everything vaporisable (sublimating samples, residual solvents) simulates water	Cannot register water of crystallisation, because of drying at 105 °C	Needs large sample quantity (1 g!), but inhomogeneities are balanced out; micronised samples (static charge) cannot be handled	Parallel analyses possible; lasts for a long time (3 h at 105 °C)
P ₂ O ₅ method Ethanol simulates water (sensor not selective); sublimation causes sensor coating	Can heat to >250 °C, i.e. no inaccurately low findings with water of crystallisation	Micronised samples (static charge) cannot be handled; homogeneity important; sublimation contaminates sensor	No automation (sampler) possible; survey analyses last for a long time, determination of water is fast
Thermogravimetry No side reaction with reagents; interpretation whether vaporisation or water with DSC; vaporisable (residual solvents, sublimation, small bp) simulate water	Types of water binding are recognised; information on decomposition; can heat to >250 °C	Very small sample quantity (homogeneity extremely important); even statically charged samples measurable	Automatable; analysis lasts for a long time due to temperature gradient
Karl–Fischer titration Sample (ketones, phosphine, etc.) can react with KF reagent	Insolubles in the solvent can cause inaccurately low findings	All samples measurable, regardless of boiling point, static charge; homogeneity	Fast; automatable via sampler, but problems with hygroscopic substances because of open vessels
KF-oven method Water selective; KF interference components (ketones) are not extracted	Inaccurately low findings possible in water of crystallisation, since only heated to 250 °C	Even statically charged samples measurable; sublimation contaminates extraction tubes; homogeneity important	Automatable (sampler); even for hygroscopic substances (closed vials); survey analyses last, determination of water fast

3.6. Hydranal standard

The water content of the Hydranal standard could be determined without difficulty with all of the methods, except for loss on drying. At the same time, direct Karl–Fischer titration did exhibit a particularly large standard deviation when compared with the other methods. Loss on drying shows inaccurate readings that are substantially too low due to the low bake-out temperature of 105 °C, which is attributable to the type of water binding in the Hydranal standard as water of crystallisation. When conducting determinations according to the P₂O₅ method and during the survey analysis (temperature ramp) in preparation for the KF-oven method, water was released from the Hydranal standard at temperatures of 130 to 210 °C. Therefore, quantitative determinations according to KF-oven method were conducted at an extraction temperature of 220 °C.

3.7. Evaluation according to categories

The analysis of the six substances show the strengths and weaknesses of the procedures. Each method has its advantages and disadvantages, which are described in Table 2 according to the categories of selectivity, type of water binding, sample properties and efficiency. Depending upon the conditions selected for the loss-on-drying method, it is possible that tightly bound water does not release, which would require higher temperatures or a lowered pressure. On the other hand, all volatile compounds do register, which also includes solvent residues or

even the substance itself, when they boil or sublimate under the appropriate conditions (temperature, pressure). An appropriate method development is therefore essential with unknown substances [11]. The same applies to thermogravimetry. Without some procedure to distinguish between the types of material loss, such as that provided by the coupled DSC in this work or for instance by coupling with a mass spectrometer [4], there can be no distinction made between water and other volatile substance and further temperature-dependent processes (vaporisation, sublimation or decomposition).

Many substances release their water only very slowly or only at high temperatures. They are, therefore, not suited for direct Karl–Fischer titration. A further problem is the low solubility of certain samples in alcohols. Other substances react with the Karl–Fischer reagents by releasing water or consuming iodine, whereby the results are falsified. Particularly aldehydes and ketones are problem groups for Karl–Fischer titration, since they react with the usual reagents to form acetals and ketals through dehydration and thus simulating the presence of water in excess. But aldehydes can enter into a further side reaction, i.e. bisulphite addition, whereby water is consumed, thus resulting in a water content that is too small [12]. This type of problems can be avoided by using the KF-oven method, since the water is first driven out and then quantified selectively during Karl–Fischer titration. The interfering matrix was thus separated. In contrast to the KF-oven method, alcohols interfere with determinations conducted by using the P₂O₅ method. Accordingly, ethanol, being highly volatile, is also driven out and causes the P₂O₅ sensor to indicate the presence of water, where none is present.

The particular advantage of the phosphorous-pentoxide method as well as that of the KF-oven method is a quantitative distinction of differently bound surface water, capillary water and water of crystallisation. The amounts of water released at the various temperatures can be determined by heating the substance with a defined temperature profile and thus enabling conclusions about the physical forces that cause the water to bind (e.g. Van der Waals forces or hydrogen bonds) [13]. In doing so, temperature limitations can cause inaccurately low findings, since the KF-oven device can only be heated to a maximum of 250 °C. The equipment used for thermogravimetry and the P₂O₅ method allowed for temperatures of up to 1000 °C or 400 °C, respectively, which is sufficient for the release of water of crystallisation.

If one takes the time factor into consideration, then the drying-cabinet method is the lengthiest. An acceleration with respect to convection heating in classical drying ovens can be achieved by microwave drying [14]. The disadvantages of covering all volatile constituents with these thermal procedures do still exist, however. The oven techniques of thermogravimetry, the P₂O₅ method and the KF-oven method take longer than does the direct Karl–Fischer titration, depending upon the bake-out program. In the case of the thermogravimetry apparatus and the KF-oven apparatus, this can be made up for through automation via a sampler. The apparatus used in the P₂O₅ method allows for introducing samples by hand, which is disadvantageous with large numbers of samples.

Except for loss on drying (sample size of about 1 g) all other procedures require a sample quantity of between 2 and 100 mg, which can be an advantage with expensive substances and a disadvantage with inhomogeneous samples. Handling statically charged samples was difficult while conducting the loss-on-drying method as well as the P₂O₅ method because the substance left the container to cling to the walls of the equipment. This did not present a problem in the other procedures that use a closed vessel.

4. Conclusions

Based on the preceding considerations, a combination of direct Karl–Fischer titration and KF-oven method is the optimal

technique for the determination of water in samples of unknown chemical and thermal behaviour. If the substance to be examined contains components that interfere with Karl–Fischer titration, causing water-content values that are too high, this can be checked by using the KF-oven method, i.e. gas extraction from the water of the interfering substance matrix and then using Karl–Fischer titration. This also provides additional information on the type of water binding. Reasons for favouring these methods are also the automation of both procedures, the good sample handling with respect to statically charged substances and simple operation (as opposed to the interpretation of DSC data).

Acknowledgements

The author wish to thank Ms. Slezewski, Mr. B athge and Mr. Kraatz for their practical support. We wish to thank the Sartorius company for lending us the WDS 400 equipment.

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