Automatic Karl Fischer Water Determination in Pharmaceuticals

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According to the European Pharmacopoeia the water content of many pharmaceuticals is determined by loss on drying in a drying cabinet or desiccator. Usually these are substances that cannot be analysed by means of direct Karl Fischer titration. Instead of applying the drying cabinet method we have used an automated Karl Fischer oven system – consisting of the 774 Oven Sample Processor and a KF coulometer – for the water determinations. The values obtained with this system all lie within the ranges specified in the Pharmacopoeia and show excellent repeatability.

Many active pharmaceutical ingredients and adjuvants contain water in an adsorbed form (surface water) or bound as a hydrate (water of crystallisation). The water content of medicaments strongly influences their quality, shelf life and stability as well as the release of the active substances. The determination of water therefore assumes great importance in pharmaceutical analysis.

The European Pharmacopoeia, 4th Edition (2002), describes various methods for determining the water content of pharmaceuticals. By far the most important method is the Karl Fischer titration. Normally the titration is carried out volumetrically (semi-micro determination). For substances with a very low water content a coulometric KF titration (micro-determination) is performed.

KF Oven Method for Difficult Samples

Many substances release their water only slowly or at high temperatures. They are therefore not suitable for a direct Karl Fischer titration. An additional problem is the low solubility of certain samples in alcohols. In these cases traditional methods recommend the use of toxic solvents to promote dissolution or alternatively extensive sample preparation procedures. Other substances undergo side reactions with the KF reagents, thus falsifying the result. The European Pharmacopoeia specifies that these types of pharmaceuticals are not to be analysed by Karl Fischer titration but by determining the loss on drying in a drying cabinet or desiccator (under vacuum if necessary). However, with this method all volatile components released at the particular temperature (e.g. impurities) are determined and not specifically the water content of the substance.

By using the KF oven method the above-mentioned problems can be avoided. The substance under investigation is heated in a tube oven and the released water is transferred by a carrier gas to the titration cell where it is determined by Karl Fischer titration.

As only the water enters the KF cell and the sample itself does not come into contact with the KF reagent, this means that unwanted side reactions and matrix effects are eliminated.

Automation Brings Clear Advantages

The 774 Oven Sample Processor allows to automate the KF oven method. In contrast to the conventional Karl Fischer drying oven, the samples are no longer introduced by means of a sample boat, but the vial technique is applied instead. The substances to be analysed are weighed directly into sample vials, which are then sealed tightly and placed in the rack of the Oven Sample Processor. For the analysis the sample vessel is moved by the turntable to the appropriate position above the oven and then lowered automatically into the heating block. At the same time a double hollow needle pierces the septum of the vial. Via the inlet needle a stream of dry carrier gas (air or inert gas) is passed through the heated sample. The carrier gas, loaded with the released moisture, then flows through the outlet needle and a heated transfer tube directly into the titration cell, where the Karl Fischer water determination takes place. Depending on the sample's water content, the determination is carried out either volumetrically or, at the trace level, coulometrically.

The automation of the Karl Fischer water determination using the 774 Oven Sample Processor brings decisive advantages:

- Strictly reproducible analysis conditions for all samples as demonstrated by the significantly improved repeatability of the results.
- Considerably increased sample throughput and therefore improved efficiency.
- Manual sample preparation is reduced to a minimum.

- Considerable savings in time.
- No contamination of the oven and titration cell; consequently there are no carryover and memory effects.
- Much lower reagent consumption as the titration solution only requires changing at infrequent intervals.
- Improved water release from the sample as the carrier gas does not just pass over the sample but directly through it.

In addition, the Oven Sample Processor allows temperature gradients to be run. Using the recorded water-release curve, it is possible to determine the optimum analysis temperature for the particular sample. The curve also allows statements to be made about the kinetics of water release as a function of temperature.

Scope of the Investigations Carried Out

Using the KF oven method, we analysed about 40 pharmaceuticals from the European Pharmacopoeia. The analyses were carried out with the 774 Oven Sample Processor in combination with a 756 KF Coulometer. The investigated pharmaceuticals were substances with a defined water content, some of which undergo side reactions with the KF reagents and therefore cannot be analysed by direct Karl Fischer titration.

As stated above, according to the Pharmacopoeia the water content of such substances must be determined by loss on drying in a drying cabinet or desiccator (under vacuum if necessary).

Instruments and Accessories

- 774 Oven Sample Processor
- 756 KF Coulometer, including KF cell without diaphragm
- 728 Magnetic Stirrer
- 6.5617.000 complementary equipment for automatic reagent exchange
- 700 Dosino
- PC with VESUV 3.0 Metrodata software for data acquisition, storage and reprocessing

Reagents

- Hydranal Coulomat AG Oven, Riedel-de Haën no. 34739
- Hydranal Water Standard KF Oven (potassium citrate monohydrate), Riedel-de Haën no. 34748
- Nitrogen as inert carrier gas

Fig. 1: The automated analysis system used for the investigations, consisting of 774 Oven Sample Processor and 756 KF Coulometer.

Analytical Procedure

Between 15 and 30 mg of the pharmaceuticals to be analysed is weighed into the sample vials, which are then hermetically sealed with PTFE-coated septa. At least a threefold determination is carried out on each substance.

Prior to each determination the complete system is conditioned until a constant low drift (approx. 10 µg/min) is attained. During this procedure the needle is located in a special conditioning vessel on the rack of the Oven Sample Processor.

In order to obtain correct results, the blank of the sample vials – i.e. the moisture adhering to the vessel walls, vial cap and septum – must be determined and taken into account when calculating the water content of the samples. This is done by analysing three empty vials at the oven temperature required for the particular sample.

The complete system is checked at regular intervals with a certified KF standard (Hydranal Water Standard KF Oven).

Determination of the Analysis Temperatures

When selecting the temperature to be used for driving off the water, the thermal stability (instability) of the particular pharmaceutical must be taken into account as well as the fact that water is only released at a sufficiently rapid rate at temperatures above 100 °C.

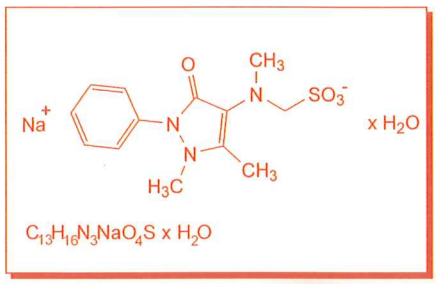

Fig. 2: Structural formula and empirical formula of metamizole sodium, present as monohydrate.

This means that the oven temperature should be chosen as high as possible to ensure short determination times, but still be 20 to 30 °C below the decomposition temperature.

The analysis temperatures are determined on the basis of the water-release curves that were recorded for all the investigated pharmaceuticals in the temperature range 50 - 250 °C. Fig. 3 shows such a water-release curve for metamizole sodium. In addition, all the pharmaceuticals were examined by means of a Kofler microscope and their melting points were determined. This instrument allows the substance to be closely observed during the heating-up and melting phases; any alterations such as colour changes, sublimation or decomposition reactions can be easily recognised.

Metamizole sodium, whose structural and empirical formulas are shown in Fig. 2, melts at 220 to 221 °C with decomposition. Water determination by direct volumetric or coulometric KF titration is not possible as the substance is oxidised completely or partially by iodine.

The water-release curve shown in Fig. 3 was recorded using a heating rate of 2 °C/min, i.e. metamizole sodium was heated from 50 to 250 °C in 100 min (= 6000 s). The red curve corresponds to the absolute amount of water released, the blue curve shows the associated drift. Both the surface moisture and the water of crystallisation are released within the time interval 0 - 1600 s (50 - 103 °C; this is indicated by the continuous increase of the red curve in this region as well as by the occurrence of the `drift peak'. The drift then falls to its original value of approx. 10 µg/min and remains virtually constant for 3800 s. Starting at 5400 s (230 °C) both curves show a steep increase. Evidently water is released by decomposition from this temperature onward.

A temperature from the central region of the plateau of the red curve (150 °C) was selected as the oven temperature for determining the water content of metamizole sodium. This ensures that the water is released quickly and completely without decomposition.

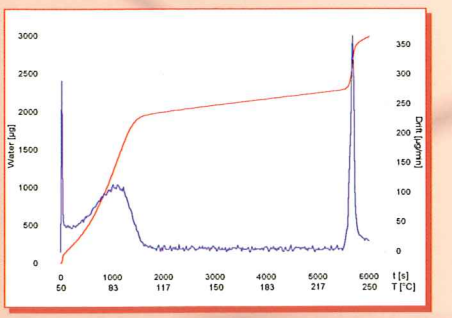

Fig. 3: Water-release curve for metamizole sodium in the temperature range 50 - 250 °C. Both the absolute amount of water and the drift are shown as functions of time and oven temperature.

By way of example, Fig. 4 shows the titration curve for morphine hydrochloride at an oven temperature of 180 °C. The red curve again corresponds to the absolute amount of water released and the blue curve to the associated drift. The three `drift peaks' show very clearly that morphine hydrochloride is present in the form of its trihydrate. It can also be seen from both curves that the substance stops releasing water after about 180 s. The subsequent slight increase of the red curve can be attributed to the low background drift (blank consumption).

It is generally recommended to use an extraction time of, for example, 5 to 10 min in order to ensure that the determination is not stopped too soon, especially if the sample releases its water of crystallisation only slowly.

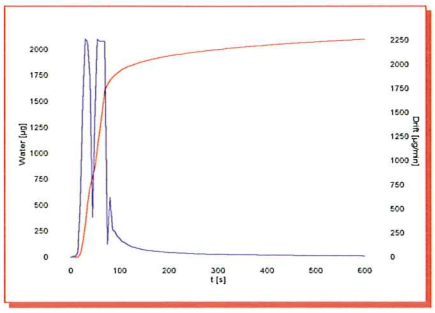

Fig. 4: Titration curve for morphine hydrochloride at an oven temperature of 180 °C. The absolute amount of water and the drift are shown as a function of time.

Discussion and Conclusions

(See Table 1.) The water contents determined with the 774 Oven Sample Processor and 756 KF Coulometer all lie within the ranges specified in the European Pharmacopoeia. The Pharmacopoeia usually gives a very wide recovery range for the loss on drying. In the case of quinine hydrochloride, for example, a range between 66.2 and 110.3% is specified, based on the theoretical (calculated) water content. In contrast, the oven system yields an excellent recovery of 96.8% for this substance. When all the investigated pharmaceuticals are considered, the recovery using the KF oven method always lies between 90 and 110%.

The repeatability of the values obtained with the oven system is also excellent. This can be seen from the relative standard deviations, which all lie between 0.30 and a maximum of 2.0%.

When compared with the drying cabinet method, the Karl Fischer water determination using the 774 Oven Sample Processor and 756 KF Coulometer offers additional clear advantages:

- Determinations that normally take several hours can be performed in 10 to 12 min. In addition, the analytical procedure is completely automated.
- A further bonus point is the specificity of the described method, in which only the water released by the substance is determined and not all the other volatile components released at the particular temperature.
- Finally the small amount of substance required is also an advantage, this aspect being particularly important when analysing very expensive pharmaceuticals. Whereas the determination of the loss on drying normally requires sample weights of 1 g, the KF oven method in combination with the KF Coulometer requires only 15 to 30 mg. Moreover, as the substance is not destroyed during the water determination it can also be used in further investigations.

Results

Table1 summarises the results of the Karl Fischer water determination using the 774 Oven Sample Processor and 756 KF Coulometer for the 15 most important substances among the 40 investigated pharmaceuticals (the water contents shown are the mean values of threefold determinations). For comparison purposes the table also contains the theoretical (calculated) water contents of the individual pharmaceuticals as well as the information about the loss on drying given in the European Pharmacopoeia.

Table 1: Results of the Karl Fischer water determination for selected pharmaceuticals from the European Pharmacopoeia, calculated water contents as well as information about the loss on drying given in the Pharmacopoeia.

Substance	Empirical formula	Molar mass [g/mol]	Melting point [°C]	Calculated water content [%]	European Pharmacopoeia		Karl Fischer water determination		
					Loss on drying [%]	Sample weight [g], temperature [°C]	Water content [%]	Relative standard deviation [%]	Temperature 774 Oven [°C]
Bupivacaine hydrochloride	$C_{18}H_{29}ClN_2O \times H_2O$	342.91	241 ... 244	5.25	4.5 ... 6.0	1.000, 100 ... 105	5.30	0.82	200
Carbidopa	$C_{10}H_{14}N_2O_4 \times H_2O$	244.25	203 ... 205	7.37	6.9 ... 7.9	1.000, 100 ... 105	7.17	0.35	150
Cyproheptadine hydrochloride	$C_{21}H_{22}ClN \times 1.5\ H_2O$	350.89	252 ... 253 (decomp.)	7.69	7.0 ... 9.0	1.000, vac. max. 0.7 kPa, 100 ... 105	7.65	1.02	150
Cysteine hydrochloride monohydrate	$C_3H_8ClNO_2S \times H_2O$	175.64	≥170 (decomp.)	10.25	8.0 ... 12.0	1.000, 24 h vac. max. 0.7 kPa	10.33	0.30	150
Dihydralazine sulfate, hydrated	$C_8H_{12}N_6O_4S \times 2.5\ H_2O$	333.31	≥160 (decomp.)	13.51	13.0 ... 15.0	1.000, 5 h vac. max. 0.7 kPa, 50	14.47	0.33	120
Ethacridine lactate monohydrate	$C_{18}H_{21}N_3O_4 \times H_2O$	361.40	≥180 (decomp.)	4.98	4.5 ... 5.5	1.000, vac., 100 ... 105	5.05	0.59	180
Histidine hydrochloride monohydrate	$C_6H_{10}ClN_3O_2 \times H_2O$	209.63	≥240 (decomp.)	8.59	7.0 ... 10.0	1.000, 145 ... 150	8.68	0.59	200
Levothyroxine sodium	$C_{15}H_{10}I_4NNaO_4 \times X\ H_2O$	798.86 (anhydrous substance)	–	–	6.0 ... 12.0	0.100, 100 ... 105	9.68	1.30	150
Metamizole sodium	$C_{13}H_{16}N_3NaO_4S \times H_2O$	351.36	220 ... 221 (decomp.)	5.12	4.9 ... 5.3	1.000, 100 ... 105	5.14	0.30	150
Metixene hydrochloride	$C_{20}H_{24}ClNS \times H_2O$	363.95	215 ... 217	4.94	4.0 ... 6.0	0.500, 138 ... 142	4.00	2.01	180
Morphine hydrochloride	$C_{17}H_{20}ClNO_3 \times 3\ H_2O$	375.85	≥200 (decomp.)	14.37	12.0 ... 15.0	0.500, 130	13.83	0.44	180
Niclosamide monohydrate	$C_{13}H_8Cl_2N_2O_4 \times H_2O$	345.13	225 ... 230	5.07	4.5 ... 6.0	1.000, 4h 100 ... 105	5.22	1.27	130
Quinidine sulfate	$C_{40}H_{50}N_4O_8S \times 2\ H_2O$	782.96	205 ... 208 (decomp.)	4.60	3.0 ... 5.0	1.000, 130	4.68	1.92	180
Quinine hydrochloride	$C_{20}H_{25}ClN_2O_2 \times 2\ H_2O$	396.91	144 ... 155	9.07	6.0 ... 10.0	1.000, 100 ... 105	8.78	1.35	135
Quinine sulfate	$C_{40}H_{50}N_4O_8S \times 2\ H_2O$	782.96	225 ... 229 (decomp.)	4.60	3.0 ... 5.0	1.000, 100 ... 105	4.63	0.33	130

decomp. = melting with decomposition
vac. = under vacuum

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