

Determination of Water Content by Capillary Gas Chromatography coupled with Thermal Conductivity Detection

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ABSTRACT

This article presents some experience obtained by applying capillary gas chromatography coupled with thermal conductivity detection (GC/TCD) to the determination of water in substances for pharmaceutical use. This technique represents a useful, orthogonal tool complementary to water determination methods based on volumetric or coulometric titration. It can also represent an alternative technique when such titrations are not applicable. This article presents the preliminary results obtained in a number of case studies where a GC/TCD procedure was applied in comparison with pharmacopoeial methods to substances with different water contents.

KEYWORDS

Capillary gas chromatography, thermal conductivity detection, water content, orthogonal technique.

1. INTRODUCTION

Volumetric (semi-micro determination) [1] and coulometric (micro determination) [2] titrations are the most common pharmacopoeial techniques for routine testing of water in pharmaceutical substances. The Technical Guide for the elaboration of monographs [3] describes gas chromatography (GC) [4] as an alternative technique for water determination. In the present case GC was coupled with thermal conductivity detection (TCD).

GC/TCD is considered of interest since it is based on a principle completely different from titration. The analyte of interest, water, is separated from other components by capillary gas chromatography and detected by a differential thermal conductivity detector [5, 6]. The water content in the sample is quantified by comparison with a reference solution [7].

Capillary GC/TCD was evaluated as it had been initially suggested by a manufacturer to determine the water content of a particular substance for pharmaceutical use. Later, it was found that GC/TCD was helpful to overcome an analytical problem encountered while applying the semi-micro method to theophylline-ethylenediamine.

Capillary GC/TCD can be used for example to confirm the applicability of the pharmacopoeial techniques to a given substance by using an orthogonal technique, which is good practice during the characterisation of primary analytical reference standards [8].

This article presents the preliminary results obtained in a number of case studies where GC/TCD procedure was applied to substances with different contents of water.

The capillary GC/TCD method was evaluated through the following steps:

- selecting the solvent to be used to solubilise the sample;
- establishing a system suitability criterion;
- testing the sensitivity of the method;
- testing the accuracy of the method on a number of substances with a known water content.

2. MATERIALS AND METHOD

2.1. Materials

Difloxacin hydrochloride trihydrate, amoxicillin trihydrate, spectinomycin dihydrochloride pentahydrate, theophylline-ethylenediamine anhydrous, and theophylline-ethylenediamine hydrate were tested in this study.

Analytical grade (> 98.5 %) dimethyl sulfoxide (DMSO), pre-dried over molecular sieves (4 Å, 2 mm) and stored under nitrogen, was used as diluent. Ultra-high quality (UHQ) water was used for the preparation of reference standard solutions.

2.2. Method

2.2.1. Apparatus and Instrumental Parameters

An Agilent 6890N gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) was used, applying the instrumental parameters reported in Table 1.

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2.2.2. Procedure

After system equilibration, the standard solutions and the system suitability mixture were injected to check the performance of the instrument.

The blank was injected to identify any peaks arising either from the solvent or from the chromatographic system. Test solutions and reference solutions were prepared and injected in triplicate.

2.3. Solvent selection

A screening was conducted to select the most suitable solvent to dissolve the substances; the choice was made taking into account the boiling point as well. DMSO was selected as it exhibited high dissolving power and sufficiently low volatility. Additional solvents were evaluated such as dioxan, dimethylformamide (DMF), formamide and tetrahydrofuran (THF). The use of DMF was discouraged because of poor peak symmetry and poor repeatability. Formamide and THF were excluded due to interfering peaks. Dioxan appeared to be a suitable alternative to DMSO.

2.4. System suitability

To identify a critical pair to be used for a system suitability test, an initial evaluation was made on 3 substances: acetone, ethanol and methanol. 5 μ L of each was mixed with 5 μ L of water, then dissolved in 10 mL of pre-dried DMSO.

The results obtained are reported in Table 2.

Methanol was selected for system suitability as it elutes at a suitable retention time and its resolution with respect to the peak due to water is within the range of 1.5 to 5.0 as recommended in the Technical Guide [3]. A typical gas chromatogram is given in Figure 1.

3. RESULTS

3.1. Difloxacin hydrochloride trihydrate

The batch of difloxacin hydrochloride trihydrate used for the study had a water content of 10.7 %, as determined previously by the semi-micro method. The limits given in the monograph are 8.0 % to 12.0 %. About 19 mg of difloxacin hydrochloride trihydrate was dissolved in 1.0 mL of DMSO with the aid of ultrasound. A clear solution was obtained, and injected in duplicate. Water was quantified using a 2.0 mg/mL solution of water in DMSO as the external standard (single-point calibration). Despite a rather high relative standard deviation (RSD = 6.4 %, $n = 3$), the amount of water found (9.6 %) was in good agreement with the value obtained using semi-micro determination of water.

3.2. Amoxicillin trihydrate

Amoxicillin trihydrate has a theoretical water content of 12.9 %. This value was confirmed by other methods, including the pharmacopoeial method. The limits given in the monograph are 11.5 % to 14.5 %. About 10 mg of a sample of amoxicillin trihydrate was accurately weighed and

dissolved in 1.0 mL of DMSO with the aid of ultrasound. The reference solutions were prepared by dissolving increasing amounts of water in DMSO to obtain concentrations in the range of 0.5 mg/mL to 4.0 mg/mL. Upon injection of the reference solutions the correlation coefficient obtained was satisfying ($r^2 = 0.995$). It is to be noted that in this case the peak shape was not optimal, therefore the height of the water peak was used for calculation instead of the area. The content of water found by GC/TCD was 13.1 % (RSD = 4.7 %, $n = 3$), which is in good agreement with the theoretical value. Again, the RSD was rather high but considered acceptable.

3.3. Spectinomycin dihydrochloride pentahydrate

Spectinomycin dihydrochloride pentahydrate has a theoretical water content of 18.1 %. The limits given in the monograph are 16.0 % to 20.0 %. About 10 mg of a batch of spectinomycin dihydrochloride pentahydrate was accurately weighed and dissolved in 1.0 mL of DMSO with the aid of ultrasound. The reference solutions were prepared by dissolving increasing amounts of water in DMSO to obtain concentrations in the range of 0.5 mg/mL to 4.0 mg/mL. The correlation coefficient obtained over that range was satisfying ($r^2 = 0.998$).

The water content found by GC/TCD was 17.6 % (RSD = 5.0 %, $n = 6$), i.e. rather close to the theoretical value of 18.1 %. This result was also in line with the value found on the same batch in a collaborative study employing the semi-micro method (17.7 %).

3.4. Theophylline-ethylenediamine, anhydrous

Anhydrous theophylline-ethylenediamine is a hygroscopic substance. A batch of anhydrous theophylline-ethylenediamine was reported to contain about 0.2 % water in the manufacturer's Certificate of Analysis.

Considering the low water content, it was decided that the quantification be made using the standard additions method [4]. A 50 mg/mL solution (5.0 g in 100 mL of DMSO) was prepared and 5 aliquots of 20 mL each were dispensed in separate volumetric flasks. Using a calibrated pipette, incremental amounts of water (5 to 20 μ L) were added to obtain a water concentration range of 0.5 % to 2.0 %. A correlation coefficient $r^2 = 0.984$ was obtained. A content of water of 1.52 % was extrapolated from the calibration curve as the x -value at $y = 0$. The difference between the result found and the value reported by the manufacturer is likely to be due to water intake during the storage of the material.

3.5. Theophylline-ethylenediamine hydrate

The water content of a batch of theophylline-ethylenediamine hydrate was 4.3 % (RSD = 9.8 %, $n = 3$), as determined by the semi-micro method in a previous study. The limits given in the monograph are 3.0 % to 8.0 %. About 50 mg of theophylline-ethylenediamine hydrate was accurately weighed and dissolved in 1.0 mL of DMSO with the aid of ultrasound. Reference solutions ranging from 0.5 % to 4.0 % of water in DMSO were injected and the calibration curve obtained showed a correlation coefficient $r^2 = 1.000$. The water content was 4.6 % (RSD = 0.9 %, $n = 6$) which is in good agreement with the value found using the semi-micro method.

4. DISCUSSION

The results obtained by GC/TCD were in agreement with the results obtained using the pharmacopoeial method in all cases.

The tentative repeatability acceptance criterion was $RSD \leq 5.0\%$, $n = 3$. This was met for a water content equal to or higher than 0.4 mg/mL. The limit of quantification was set at 0.4 mg/mL water (i.e. 2.0 % for a concentration of 20 mg/mL). This was substantiated by the finding that at a concentration of 0.04 mg/mL, the repeatability was poor ($RSD > 30\%$, $n = 3$).

Based on the above, the capillary GC/TCD method can be considered sufficiently repeatable and accurate to quantify water above 0.4 mg/mL.

If it appears necessary to increase the sensitivity, the sample concentration is to be increased, obviously as long as the substance remains soluble in DMSO.

The method is of limited applicability to substances with low solubility in DMSO. To circumvent this problem, alternative solvents were evaluated. Hydranal-Coulomat AG (containing diethanolamine, imidazole, methanol and sulfur dioxide) was tested but it was not considered suitable due to the presence of interfering peaks, as shown in Figure 2.

DMSO is known to be hygroscopic. Solvents of lower hygroscopicity, e.g. dioxan, dimethylformamide, formamide and tetrahydrofuran, were therefore considered. Dioxan appeared to be a suitable alternative to DMSO, whereas the other solvents showed a number of interfering peaks.

The amount of water in the solvent (DMSO) can clearly affect the quantification. As previously underlined, DMSO is hygroscopic and the water uptake – for example when using bottles that were opened several days before sample preparation – is unlikely to be negligible for the scope of this determination. To minimise the interference, the water content should be checked prior to use and a limit of 30 $\mu\text{V}\cdot\text{s}$ has been set as the maximum acceptable water peak area in the solvent. It is recommended that either unopened DMSO bottles be used or molecular sieving be introduced.

The quantification made using an external standard provided results considered satisfactory in terms of accuracy and precision. The external procedure is usually the preferred option as it is simpler than the standard additions procedure in terms of sample preparation. Despite the drawback that it is time- and material- consuming, the standard additions method can become a more suitable approach when the water content is low or when the material to be tested is hygroscopic. In the present case, the test material was dissolved in DMSO and then spiked with incremental quantities of water.

Based on the present study, the following acceptance/suitability criteria are recommended for the water determination by GC/TCD:

- clear separation between the peak due to water and any other peak;
- relative retention of methanol in the conditions used should be 1.06 ± 0.2 ;
- system suitability is met when:
 - the resolution between methanol and water is equal to or greater than 2.0;

- in case no baseline separation is obtained, the peak-to-valley ratio must be at least of 4.0;
- the symmetry factor of the peak due to water must not exceed 2.5;
- the correlation coefficient of the 6-point calibration line is at least 0.98;
- the RSD of the response (area or height) of the reference peak calculated over 3 consecutive injections of the reference solution does not exceed 5 %.

5. CONCLUSIONS

A capillary GC/TCD method for the determination of water in pharmaceutical substances has been tested on a number of samples. This method seems sufficiently accurate and repeatable and it appears to be a useful, orthogonal method complementary to the volumetric or coulometric titration and an alternative technique when such titrations are not applicable.

Appropriate measures have been developed and are recommended to enhance the sensitivity of the method, such as using the highest possible sample concentration in DMSO allowed by solubility and using unopened DMSO bottles to minimise interferences and maximise the sensitivity towards the peak due to water.

The above method has to be validated for any specific use. Nonetheless, a set of system suitability criteria is proposed to assist users employing this method in getting an independent confirmation of results obtained by pharmacopoeial methods. The recommendations obtained from this study may be considered for future inclusion in the Technical Guide.

6. REFERENCES

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Table 1 – GC/TCD parameters

Column type	Cross-linked polycyanopropylphenylsiloxane 6 % / polydimethylsiloxane 94 %
Column length (m)	30
Internal diameter (mm)	0.32
Film thickness (µm)	1.8
Carrier gas	Helium for chromatography R
Flow rate (mL/min)	2.2
Detector temperature (°C)	250
Injector temperature (°C)	250
Injection mode	Splitless (purge time 0.1 min, purge flow rate 60 mL/min)
Injection volume (µL)	2
Detector type	Thermal conductivity (TC)
TCD parameters:	
- reference flow rate (helium) (mL/min)	30
- make-up flow rate (constant) (mL/min)	2
- switch time (min)	0
- polarity negative	OFF
Oven temperature	0 - 3 min: 80 °C; isotherm 3 - 9 min: 80 °C to 200°C; rate: 20 °C/min 9 - 12 min: 200 °C; isotherm

Table 2 – Critical pair for a system suitability test

Sample	Water peak area ¹ (µV·s)	Solvent peak area (µV·s)	Retention time (min)		Relative retention	Resolution
			Water	Solvent		
Blank (DMSO only)	19.7	n/a	1.70	8.06	n/a	n/a
Water/methanol	25.1	24.3	1.70	1.81	1.07	2.9
Water/ethanol	18.1	35.0	1.70	2.03	1.20	8.0
Water/acetone	15.9	15.5	1.70	2.22	1.31	12.2

¹ area of the peak due to water corrected by subtracting the area of the blank (DMSO).

n/a = not applicable

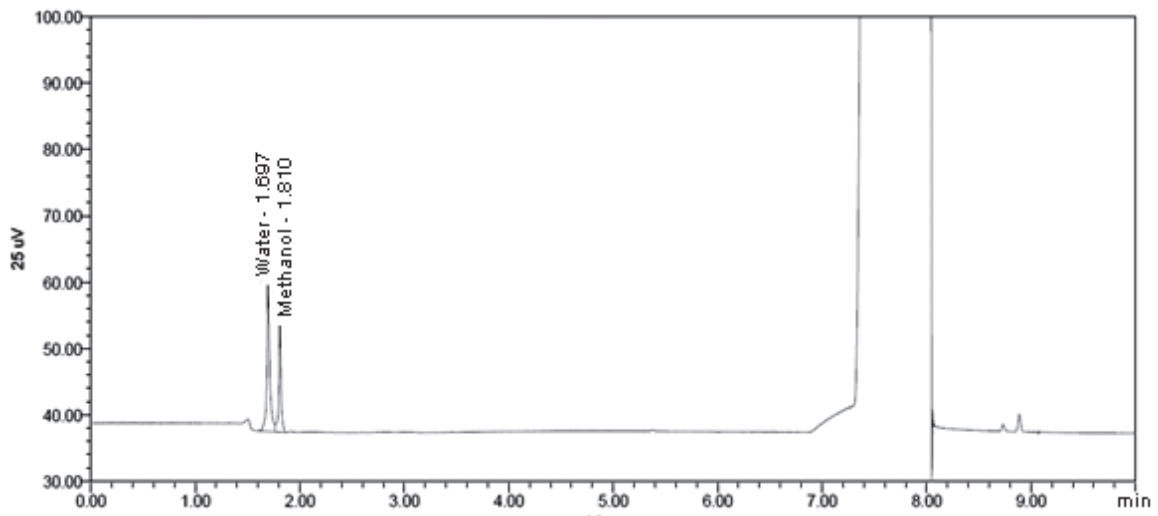


Figure 1 – System suitability test: typical gas chromatogram

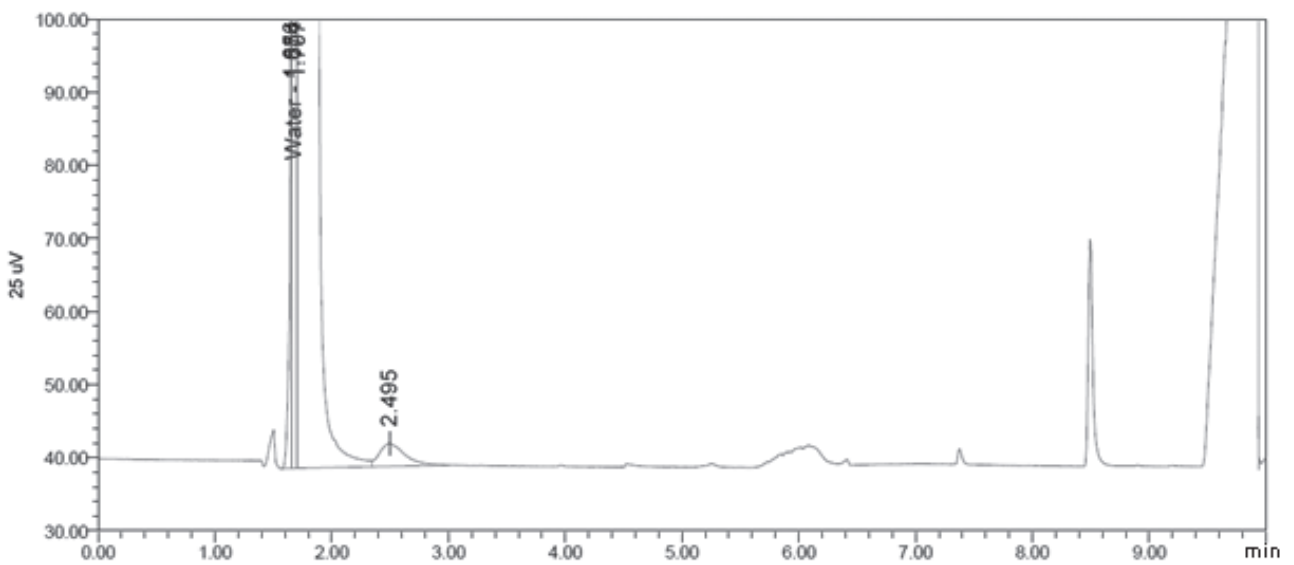


Figure 2 – GC chromatogram obtained using Hydranal-Coulomat AG as solvent