# Application of Thermogravimetry to Water-Content Determinations of Drugs[1)]

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**Water-content data for various drugs, estimated on the basis of weight decrease in a loss on drying (LOD) test or determined by the Karl–Fisher method (KF), were compared with values obtained with thermogravimetry (TG). TG/mass spectroscopy was also utilized for identification of volatile materials during the weight-loss process. The results suggest that the TG method can be applied to the determination of water content in drugs if their volatile contaminant is only water or the major volatile constituent is water, and that TG can be used as a substitute for the LOD test in cases, where expense or some other factor restrict the available sample size of a drug. It was further indicated that TG can be utilized for some drugs to which the KF method cannot be applied due to their insolubility in KF reagents.**

**Keywords** thermal analysis; thermogravimetry; Karl–Fisher aquametry; water content; United States Pharmacopeia; Japanese Pharmacopoeia

Thermal analysis involving differential scanning calorimetry (DSC), differential thermal analysis and thermogravimetry (TG) has been utilized to analyze a variety of drugs.[2–5)] Pharmaceutically related organic and inorganic hydrates have previously been studied by using TG and DSC techniques that can discriminate between free and bound water.[4)]

Thermal analysis is specified in the United States Pharmacopeia (USP) XXII[5)] and the British Pharmacopoeia 1993.[6)] In USP XXII, TG and DSC are recommended as a means of loss on drying (LOD) test, namely, the determination of volatile matter of any kind that is driven off under the specified conditions, and as a characterization method for polyethylene containers, respectively.[5)]

Residual moisture limits have been set for freeze-dried biological products[7,8)] and the stability, viability and potency of such materials are favored by the maintenance of a low content of residual water.[9)] There is a particular need to validate moisture determining methods that can be applied to samples that are small and not easily handled by the regulation (gravimetric) method.[8,10)] In these cases, TG and Karl–Fischer aquametry (KF) have been applied to the measurement of residual water in freeze-dried biological products.[11)] When drugs are expensive and/or only small amounts are available for testing, TG has an advantage in the analysis of their moisture content since this method requires only a few mg.

In the Pharmacopoeia of Japan (JP) XII, thermal analysis is not specified as a general test, and the water content of drugs is to be determined using the LOD test as a total of residual water and volatile-matter in some cases. The KF is also accepted for use as a water-determination method for many drugs.

The combination of TG and mass spectrometry (TG/MS)[12)] has proven effective in problem solving related to the composition analysis of biological products by providing precise TG heating conditions and weight-loss information, along with mass spectral identification of volatile matter driven off during the weight loss process.[7)]

In the present study, a variety of drugs including steroid hormones, vitamins and cardiac glycosides were selected as representative samples, and their water content, estimated on the basis of weight loss using the LOD test or determined by KF, was compared with weight-loss values obtained with the TG method. The TG/MS technique was also utilized for the identification of volatile materials during the weight-loss process. The potential for application of the TG method as a replacement for LOD and KF methods is also discussed.

## Experimental

**Materials** Disodium edetate (lot No. 431739) was purchased from Katayama Chemical Industries Co., Ltd., Osaka, Japan. Vinblastine sulfate (lot No. 58454.00) was obtained from Kyorin Pharmaceutical, Ltd., Tokyo, Japan. Glycyrrhizinic acid (lot No. WDM7409) and sodium tartrate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Dexamethasone *m*-sulfobenzoate (lot No. 7J1561) was obtained from Nihon Tenganyaku Research Institute Co., Ltd., Nagoya, Japan. G-Strophanthin (product reference standard in Sandoz, No. 192) was obtained from Sandoz Pharmaceuticals, Ltd., Tokyo, Japan. Other drugs follow the Japanese Pharmacopoeia Reference Standards (JP-RS) or the National Institute of Hygienic Sciences Reference Standards (NIHS-RS). These include thiamine hydrochloride RS (control Nos. 8411 and 766), methotrexate (No. 771), lanatoside C (No. 781), hydrocortisone sodium phosphate (No. 891), folic acid (No. 743), betamethasone sodium phosphate (No. 881), deslanoside (Nos. 781 and 721), cyanocobalamin (Nos. 804 and 773) and lysozyme RS (Nos. 711 and 811).

**Measurements** In order to minimize the scattering of measured values due to the absorption of moisture by samples with the passage of time, measurements by TG, KF and the LOD test were performed at the same time where possible.

TG: TG was carried out at the heating rate of 5 °C/min using a Shimadzu TGA-50 thermogravimeter equipped with a Shimadzu TA-50WS thermal analyzer. An analysis program, ver. 1.01, supplied by Shimadzu was used to analyze the recorded data. Weight losss was observed from room-temperature to about 230 °C. The temperature scale of the TG apparatus was calibrated using the Curie point of metal nickel (purity, 99.99%) as 353 °C, the mass scale being calibrated by the use of standard weights. Dry nitrogen gas was used as the atmosphere and the flow rate was controlled at 20 ml/min. The desired weights of samples were added to aluminum pans (6 mm inner diameter and 5 mm height) without sealing, and the measurements were then performed immediately.

Reference measurements were performed using empty pans, and all data was corrected by the subtraction of the reference from observed

values.

KF: Titrimetric determinations of water were performed at room temperature (about 20 °C) using a Hiranuma AQ-6 Karl–Fisher moisture content meter equipped with a coulometric titration system. The Karl–Fisher reagent, Hydranal, was purchased from Riedel-de Haën (Hannover, Germany). Sample amounts containing more than 100 $\mu$g water were taken, and measurements were carried out immediately in a low-moisture atmosphere.

LOD Test: Measurements were carried out for deslanoside and cyanocobalamin, lysozyme and disodium edetate according to the procedures for the LOD test in the JP XII, the Japanese Pharmaceutical Codex 1993 (JPC), and the Japanese Standards of Food Additives Commentary ed. 6 1992, respectively. Weight loss was measured after the following drying procedures: for deslanoside (0.5 g, 60 °C, 4 h), for cyanocobalamin (0.05 g, 100 °C, 4 h, in a vacuum using phosphorus pentoxide as a desiccant); for lysozyme (0.1 g, 60 °C, 4 h); for disodium edetate (1 g, 90 °C, 1 h).

TG/MS: TG/MS data were obtained using a Shimadzu TGA-50 thermogravimeter interfaced with a Shimadzu GCMS-QP1100EX mass spectrometer. In the TG system of the TG/MS apparatus, the measurement conditions were the same as for the TG measurement alone except for the gas flow rate at 50 ml/min. The temperature at the transfer-line of gas was kept the same as in the furnace of the TG apparatus. The MS measurements were carried out under the following conditions: ionization potential, 70 eV; ionizing current, 60 $\mu$A; source temperature, 250 °C; sampling frequency, 6 s/scan; and mass region for the detection, 10—300. The spectra were recorded in the peak-finder mode saving all spectra.

## Results and Discussion

Figure 1a shows the typical weight-loss curve (TG-curve) and its derivative (DTG-curve) as a function of temperature for sodium tartrate. In the initial stage, the weight loss is very small, followed by an increase with the rise in temperature. After a final steep drop, the weight loss stops at about 160 °C and the weight becomes constant in spite of increasing temperature to approximately 240 °C. In most drugs, such a plateau is observed in their TG curves. The abrupt decrease in the weight above 240 °C can be ascribed to degradation of the material itself. The difference between the initial and final flat portions is evaluated as the weight-loss value.

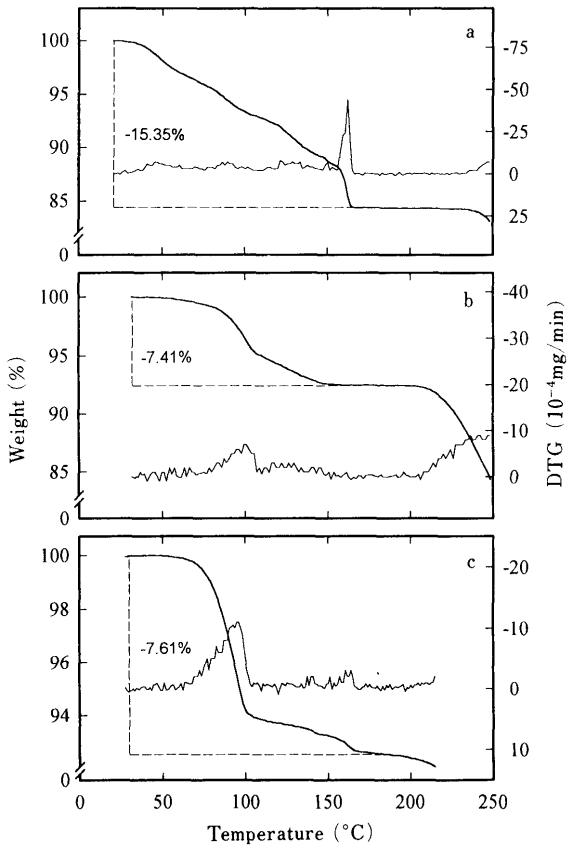


Fig. 1. Typical TG- and DTG-Curves Observed with TG

a, sodium tartrate (sample amount, 5.39 mg); b, folic acid (3.56 mg); c, hydrocortisone sodium phosphate (8.25 mg). Numerals in these figures indicate the weight loss percent in each case. The weights at the beginning of measurement are represented as 100%. Thick lines, TG-curves; thin lines, DTG-curves (derivatives of TG-curves).

The weight loss of sodium tartrate as a function of sample weights was assayed using the minimum sample amount of sodium tartrate for our apparatus, the plots being presented in Fig. 2. With sample amounts above about 1.5 mg, the weight-loss values were found to be constant. However, below 1.5 mg, the values decrease abruptly with a reduction in sample weight. This can be ascribed to the influence of buoyancy and convection currents,[13] so sample weights at which these influences can be ignored are required for the accurate measurement of weight loss. TG is also affected by the particle size of samples, the surface of crystals and the crystal forms, and these details were discussed by Paulik *et al.*[14]

For sodium tartrate, the average weight-loss is 15.40±0.10% (average±S.D., $n=8$) at amounts above 1.5 mg.

Figure 1b shows the typical TG curve and its derivative (DTG) for folic acid. The weight loss stops at about 190 °C. The average weight-loss evaluated from four runs at sample amounts of 3.5—5 mg was 7.57±0.03%. The moisture content was determined by KF to be 7.65±0.10%, from three runs with sample amounts of 6—7 mg. The weight loss/water content values are therefore essentially equal.

Figure 1c depicts the typical TG and DTG-curves for hydrocortisone sodium phosphate. There are three temperature regions at which abrupt decreases in weight were observed: at 70—110 °C, at 110—150 °C and at 150—190 °C. TG can usually distinguish between absorbed solvents in various states or with a variety of bound energies.[4] This, therefore, suggests variation in the water and/or other solvents contaminating the sample

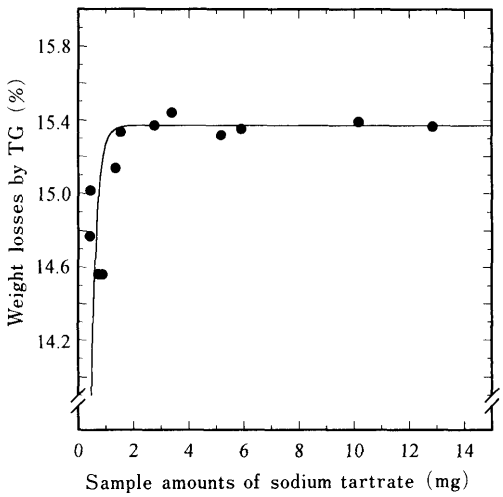


Fig. 2. Weight Losses of Sodium Tartrate as a Function of Sample Amount Using the TG Method

Filled circles, observed points; the line curve was arbitrarily drawn through the observed points.

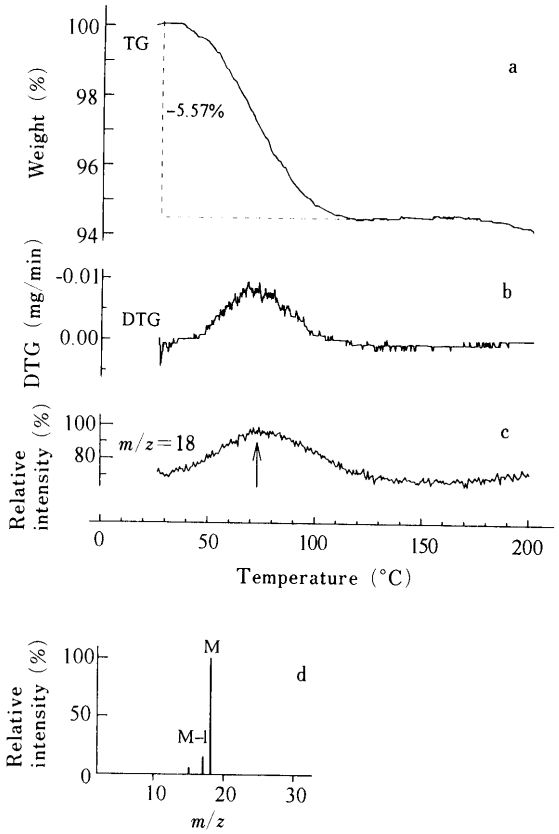


Fig. 3. Typical TG, DTG and Ion Intensity Curves, and a Mass Spectrum for the Peak of the Ion Intensity Curve for Lysozyme

a, TG; b, DTG (derivative of the TG curve); c, ion intensity at $m/z=18$; d, mass spectrum of the peak (indicated by the arrow) of the ion intensity curve. Sample amount is 6.20 mg.

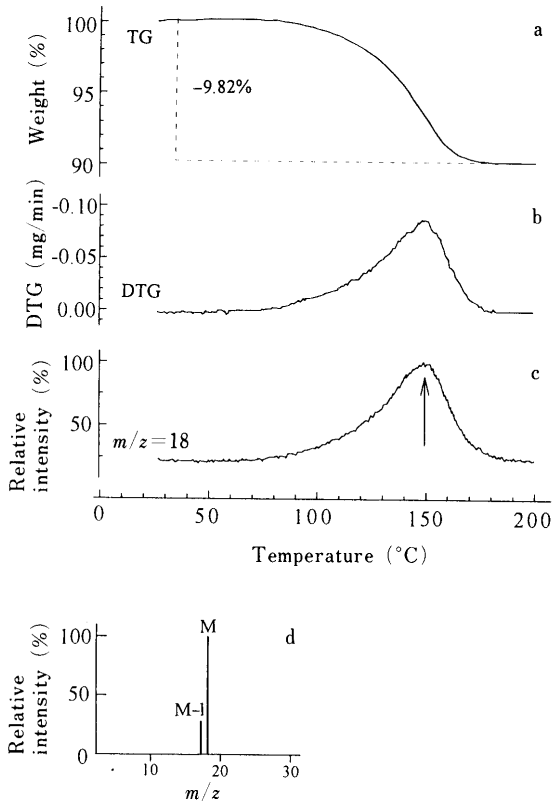


Fig. 4. Typical TG, DTG and Ion Intensity Curves, and a Mass Spectrum for the Peak of the Ion Intensity Curve for Disodium Edetate

a, TG; b, DTG (derivative of the TG curve); c, ion intensity at $m/z=18$; d, a mass spectrum of the peak (indicated by the arrow) of the ion intensity curve. Sample amount is 10.20 mg.

driven off at the three different stages. Such differences in the slopes of decrease, observed for sodium tartrate, folic acid and also vinblastine sulfate, presumably represent different existing states of volatile constituents being lost at different rates.

The average weight-loss evaluated from three runs with sample amounts of 8—10 mg was $7.44\pm0.02\%$ for hydrocortisone sodium phosphate. On the other hand, the water content was determined by KF to be $7.28\pm0.04\%$ from three runs for sample amounts of 14—20 mg. Thus, the TG value is slightly larger than the KF moisture content, further suggesting that hydrocortisone sodium phosphate may contain trace amounts of solvents other than water. However, TG/MS identification of volatile materials during the TG process revealed only $H_2O$ , and the content of other contaminating solvents was calculated to be only 0.16%. This indicates that the surplus solvent content is too small for detection with our TG/MS equipment.

Figure 3a shows a typical TG-curve for lysozyme. Moderate decreases in weight were observed, with an average weight-loss evaluated to be $5.72\pm0.13\%$ from three runs at amounts of 4—5.5 mg. On the other hand, the weight loss observed by LOD was found to be $4.37\pm0.06\%$ from three runs with sample amounts of 79—95 mg. The fact that the TG value was significantly larger than that gained according to LOD again indicates the presence of other volatile of constituents.

Figure 3c represents the ion intensity in $m/z=18$ ($H_2O$) as a function of temperature using the TG/MS apparatus. This spectrum has a peak at about 70 °C and corresponds to the DTG-curve shown in Fig. 3b. The mass spectrum at the peak (indicated by the arrow in this figure) is depicted in Fig. 3d, indicating that it contains fragments originating only from $H_2O$. This suggests that the sample contained only water as a volatile material, and that thermal decomposition of the drug did not take place.

The LOD test involves a drying procedure for 4 h at 60 °C, but dose not require drying in a vacuum or the use of phosphorus pentoxide as a desiccant. As shown in the TG-curve, a decrease in weight continued to only about 110 °C. Therefore, a TG value significantly larger than that gained for LOD could be ascribed to the LOD procedure at low temperature for a short period. Similar results were observed for deslanoside.

The application of KF to lysozyme was attempted. Lysozyme proved, however, to be insoluble in the KF reagent composed of methanol and chloroform as its main components, and therefore it was difficult to determine the lysozymal water content.

Figure 4 illustrates TG (Fig. 4a), DTG (Fig. 4b) and ion intensity (Fig. 4c) curves for disodium edetate, another typical drug for which the KF method can not be applied because of the drugs insolubility in the KF reagent. The peak on the DTG curve corresponds to that on the curve for ion intensity at $m/z=18$. The mass spectrum of this latter is shown in Fig. 4c, demonstrating fragments originating only from $H_2O$. These results suggest that the volatile material driven off during the weight-loss process

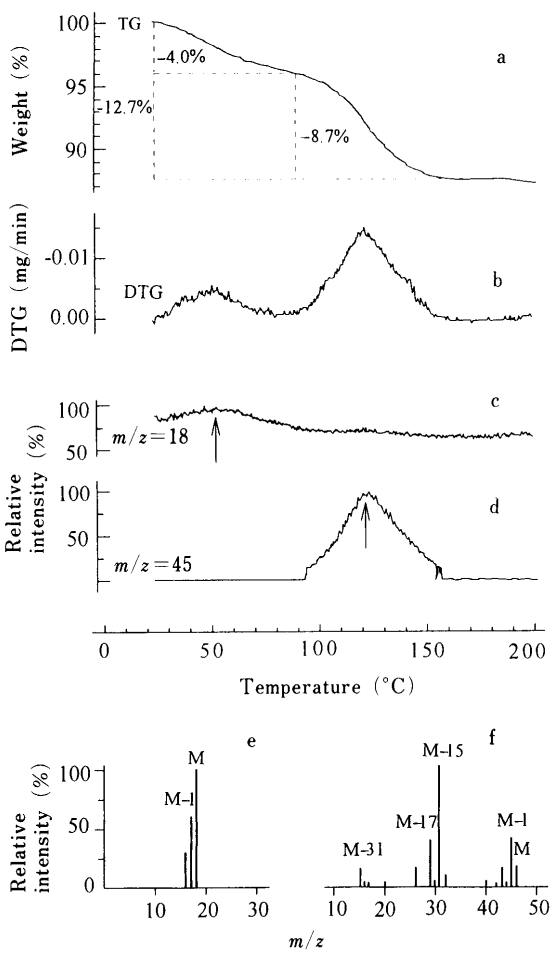


Fig. 5. Typical TG, DTG and Ion Intensity Curves, and Mass Spectra for the Peaks of the Ion Intensity Curves for Vinblastine Sulfate

a, TG; b, DTG (derivative of the TG curve); c and d, ion intensity curves at $m/z=18$ and 45, respectively; e and f, mass spectra of the peaks (indicated by arrows) of the ion intensity curves c and d, respectively. Sample amount is 5.00 mg.

was only water. The average weight loss evaluated from three runs with sample amounts of 10—17 mg was 9.83±0.04%. Disodium edetate has two molecules of crystallized water and the theoretical water content corresponds to 9.67%. The value is therefore in good agreement with the weight loss (%) estimated using the TG method.

Figure 5 presents TG (Fig. 5a), DTG (Fig. 5b) and ion intensity (Figs. 5c and 5d) curves for vinblastine sulfate. Two temperature regions with a gradual change in the rate of decrease in weight were observed. The average weight loss was evaluated as 12.28±0.38% from four runs with sample amounts of 3—6 mg. In clear contrast, the water content estimated by KF was 4.70±0.15% from three runs with sample amounts of 5—6 mg. The fact that the weight loss estimated by TG is larger than the KF water content indicates that vinblastine sulfate probably contains appreciable quantities of residual volatile-components other than water.

The DTG-curve shows two distinctive peaks at about 50 and 120 °C. The peak at about 50 °C on the DTG-curve corresponds to the peak on the curve for ion intensity at $m/z=18$ (Fig. 5c), and the other peak at about 120 °C agrees with that at $m/z=45$ (Fig. 5d). Mass spectra for the respective peaks are shown in Figs. 5e and 5f, indicating

Table I. Weight Losses Estimated by TG and LOD Methods, and Water Content Determined by KF Aquametry

| No.[a] | Drugs | Weight loss (%) by TG | Weight loss (%) by LOD | Water content (%) by KF |
|---|---|---|---|---|
| | | Average±S.D. ($n=3$) | | |
| 1 | Betamethasone sodium phosphate | 13.10±0.24 | nd | 13.37±0.06 |
| 2 | Cyanocobalamin | 8.63±0.09 | nd | 8.79±0.09 |
| 2′ | | 11.71±0.05 | nd | 11.69±0.15 |
| 2″ | | nd | 13.17±0.10 | 13.96±0.18 |
| 3 | Deslanoside | 3.75±0.12 | nd | 4.42±0.12 |
| 3′ | | 7.84±0.05 | 6.69±0.34 | 7.44±0.15 |
| 4 | Dexamethasone *m*-sulfobenzoate | 3.17±0.07 | nd | 3.14±0.12 |
| 5 | Disodium edetate | 9.83±0.04 | nd | dif |
| 5′ | | 9.81±0.06 | 10.10±0.05 | dif |
| 6 | Folic acid | 7.57±0.03 | nd | 7.65±0.10 |
| 7 | Glycyrrhizinic acid | 7.06±0.12 | nd | 6.33±0.12 |
| 8 | G-Strophanthin | 4.35±0.07 | nd | 4.55±0.30 |
| 9 | Hydrocortisone sodium phosphate | 7.44±0.02 | nd | 7.28±0.04 |
| 10 | Lanatoside C | 7.25±0.12 | nd | 7.02±0.02 |
| 11 | Lysozyme | 5.72±0.13 | 4.37±0.06 | dif |
| 12 | Methotrexate | 6.61±0.27 | nd | 5.83±0.06 |
| 13 | Sodium tartrate | 15.40±0.10[b] | nd | 14.15[c] |
| 14 | Thiamine Hydrochloride | 2.99±0.07 | nd | 3.27±0.03 |
| 15 | Vinblastine sulfate | 12.28±0.38 | nd | 4.70±0.15 |

*a*) Numbers correspond to the numbers in Figs. 6a and 6b. Signs, one or two primes, on the numerals in the cell shows the individual measurements. *b*) Average from eight runs ($n=8$). *c*) Average from two runs ($n=2$). nd, not done; dif, difficult to measure.

that the fragments originated from $H_2O$ and ethanol, respectively. These results suggest that water is released from the drug up to about 90 °C, and then ethanol evaporation follows.

When a distinct TG curve, as in this case, is evaluated, the contents of the contaminating vapor components can be estimated separately using line-drawing on the TG curve, as shown in Fig. 5a. In this case, the results gained indicated that the sample contained 4.0% water and 8.7% ethanol. The residual ethanol can be ascribed to the solvent used in the recrystallization.

Table I summarizes the weight loss values estimated by TG and LOD, and water content determined by KF. Figures 6a and 6b show correlative comparisons of weight loss values from TG with those from LOD and with KF water content, respectively. In both cases, a good correlation between the sets of data for drugs containing water as a volatile contaminant is evident, except in vinblastine sulfate, which contains about 8.7% ethanol as a residual solvent other than water, as mentioned above. This provides direct support for the conclusion that the TG method can be applied to the determination of water content of drugs if they contain water as the volatile constituent or as a major volatile component.

As is widely known, the LOD and/or KF methods specified in the JP XII require relatively large amounts of sample, usually above 0.5 g. The amount of drugs required for the LOD and/or KF methods are 0.05—1 g, and therefore, relatively large. The TG method has the

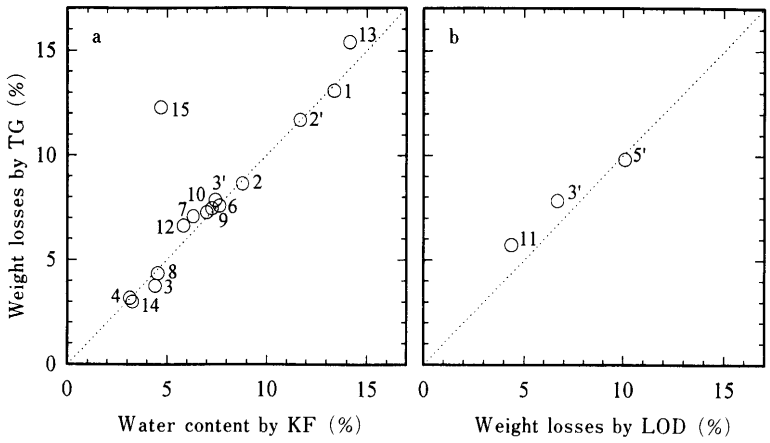


Fig. 6. Comparison of Values for Weight Loss or Water Content Obtained with the TG, LOD and KF Methods

a, comparison between TG weight loss and KF water content; b, comparison of weight loss values between TG and LOD. Numbers affixed to symbols correspond to the drug number in Table I.

advantage that only a small amount of sample, for example 2—10 mg, is required to achieve reliable measurements. Therefore, TG can be used as a substitute for the LOD test for the water-content determination of drugs for which only limited quantities of sample are available due to expense or some other factor. Furthermore, TG can be utilized for some drugs, such as lysozyme and disodium edetate for which the KF method cannot be applied because of the drug's insolubility in the KF reagents. Another advantage of TG is the possibility of the simultaneous and separate estimation of different volatile substances, one of which would be water.

**Acknowledgements** A part of this work was supported by the Society of Japanese Pharmacopoeia. We are grateful to Mr. Mitsuru Ohta and Mr. Shin-ichi Kawano of the Analytical Application Center, Shimadzu Corporation, for helpful criticism during this work. We thank Dr. Malcolm A. Moore for comments on the manuscript.

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