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Determination of water in penicillins using fast Karl Fischer reagents and electronic end-point optimization

JÖRGEN LINDQUIST

Astra Läkemedel AB, Research and Development Laboratories, Pharmaceutical Analysis, S-15185 Södertälje, Sweden

Abstract: When using conventional Karl Fischer reagents for titration of water in penicillins, decomposition products (i.e. penicilloic acid) are shown to be partly co-titrated. Water in ampicillin, bacampicillin, carboxybenzylpenicillin and cloxacillin was titrated with slow and fast reagents and the differences in the results obtained were compared with the content of penicilloic acid determined mercurimetrically. A simple electronic control unit has been developed to optimize the speed of titration.

Keywords: *Karl Fischer titration; ampicillin; bacampicillin; carboxybenzylpencillin; cloxacillin; penicilloic acid.*

Introduction

During the last decade some important contributions in the reagents used for Karl Fischer titrations of water have been proposed. In 1974 Cedergren [1] showed that the concentration of excess pyridine had no measurable effect on the rate of the main reaction, and that the reaction was first-order with respect to iodine, sulphur dioxide and water. In a later paper he showed [2] that in a mixture of pyridine–formamide (60:40, v/v) the reaction rate was increased by a factor of 100. Verhoef *et al.* [3–5] showed that pyridine can be replaced by other buffer components with higher pK_a values. The reason for this is said to be the increased concentration of monomethylsulphite ion, which is the species reacting with iodine in the presence of water.

A new reagent is now on the market, where pyridine is replaced by methanolic acetate buffer (Reaquant). In 1980 Scholtz proposed replacing pyridine by other amines [6–7]. He found that stronger bases gave less stable reagents with unstable titration end-points. Very good results were obtained, however, when combining the amines with excess sulphur dioxide. Another reagent is consequently on the market, where pyridine is replaced by diethanolamine (Hydranal).

The two new reagents are claimed to have very high reaction rates, stable titration end-points and unlimited storage stability when solvent and titrant are stored as two separate solutions. Two other commercially available reagents, with specified composi-

tion, are claimed to be fast, *viz.* the methanol-free 'Karl Fischer Solution Rapid' (Merck) and the pyridine-free Merck 9241, 9243.

The advantages of using fast reagents are obvious. Apart from shorter titration time, the risk of interfering side reactions will be reduced. This has already been shown by using the flow injection technique with a short reaction time [8].

In the present study fast reagents have been used for titration of water in penicillins, with an electronic control unit specially developed to optimize and shorten the time of titration.

Materials and Methods

Titrations using conventional Karl Fischer (KF) reagents were performed using the Karl Fischer–Automat E 547 of Metrohm AG, Herisau. The other titrations were performed with a Metrohm Multi-Dosimat 655, using a 10-ml burette. This was controlled manually by using an amperometric end-point detector or by the control unit developed. Air was continuously pumped with an aquarium pump through two drying towers, containing silica gel and molecular sieve 4A respectively, and then through the titration cell waste via an outlet tubing, in order to avoid contamination by water vapour [9]. A Brand 'Dispensette' was used to add the 20 ml of KF solvent into the cell. After the titrations the used solution was pumped out by pushing the outlet tubing to the bottom of the cell.

Reagents

Conventional KF Reagent (Merck 9246, 9247; Darmstadt); Karl Fischer Solution Rapid (Merck 9245); Hydranal Solvent and Titrant (Riedel-de Haen 34800, 34801).

The penicillins were obtained from Astra Läkemedel AB (Södertälje, Sweden). Penicilloic acid was determined by mercurimetric titration [10]. The pH* was measured directly in the methanolic solvents using a glass electrode.

Electronic control unit

The control of the titration is based on two potential functions and an amperometric end-point detector. A sawtooth waveform (e_1 in Fig. 1) is continuously generated when a potential ramp (e_2) is started at the beginning of the titration. The motor-driven burette delivers titrant when $e_2 > e_1$ and the detector current (i_m) is below the end-point value (i_e). This gives a slow start to the titration, which is important in order not to overtitrate small samples.

After this careful start the burette will dose continuously at an adjustable high speed, until the detector senses the approaching end-point when titrant is delivered in smaller and smaller portions up to the end-point. This is achieved electronically by summing the detector signal and the sawtooth signal, which is then raised to a higher level, so that its peaks are crossed by e_2 to give increasingly narrow zones where $e_2 > e_1$.

At the end-point the burette is stopped and the ramp e_2 proceeds in a negative direction, reaching potential s after 20 s indicating that the titration is finished. If the detector signal drops below i_e during the 20-s period, e_2 will turn positive and eventually cross a peak of e_1, whose width is narrower (and thus pulses a smaller portion of titrant) the longer the time that elapses from the end-point stop. The electronic circuit diagram is illustrated in simplified form in Fig. 2 (a more detailed circuit diagram is available from the author on request).

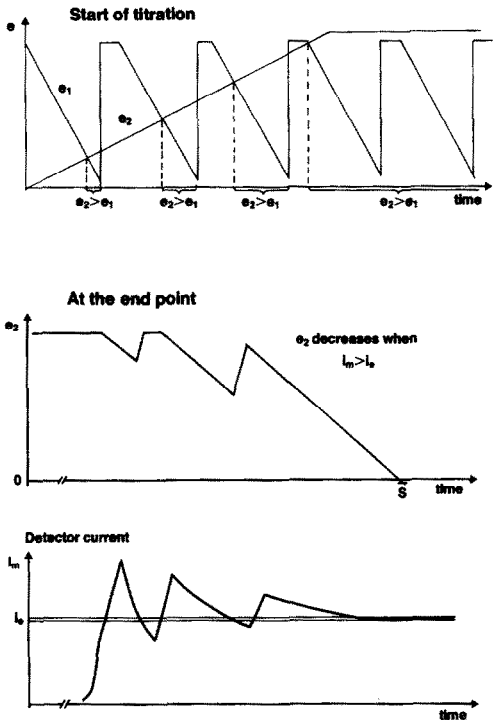


Figure 1
Functional diagram of the titration control system. The motor-driven burette is on when $i_m < i_e$ and $e_2 > e_1$; i_m = detector current; i_e = current at equivalence point; e_1 = sawtooth waveform voltage; e_2 = ramp voltage.

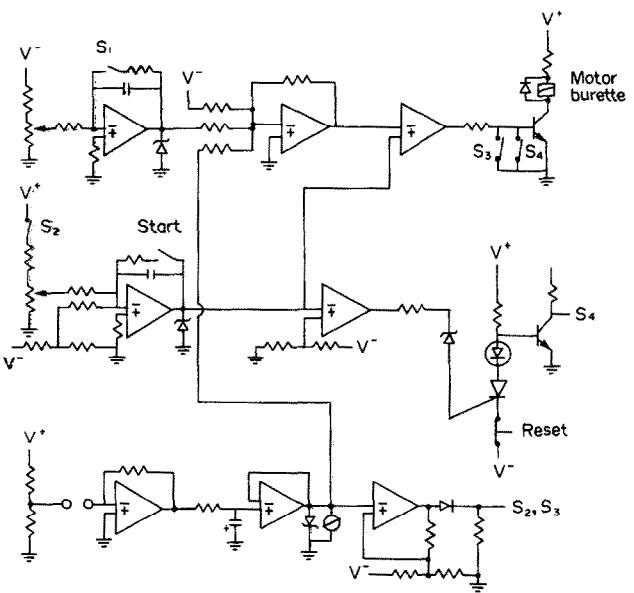


Figure 2
Diagram of the electronic control unit. S_1–S_4: 4016 B Quad bilateral switches; operational amplifiers: LM 324 Quad units; clock for S_1: NE 555.

Results and Discussion

Evaluation of the electronic control unit

The control unit was tested by titrating liquid samples (4% v/v water in methanol) to measure the precision at different speeds of titration. The samples were added using a Unimetrics 250-μl syringe provided with an adapter (2300) for maximum reproducibility. Its calibrated volume was 114.2 ± 0.2 μl (n = 10). Solid samples known to give 'creeping end-points' (e.g. aminobenzylpenicillin) with conventional KF reagents were also titrated to study the precision.

Table 1 shows that the accuracy and precision decreased only slightly at higher speeds of titration and that there was no overtitration, even at a speed as high as 12 ml/min. For larger samples (40–45 mg of water) a relative standard deviation of 0.1% was obtained at a speed of 3.5 ml/min.

Table 1
Titration of water in methanol. Five titrations were performed at each titration speed, using Hydranal titrant and solvent

Speed of motor-driven burette (ml/min)	Titrant* (ml±σ)	Titration time† (s±σ)	Water content (mg±σ)
0.8	0.966±0.005	68±5	4.54±0.02
2.0	0.970±0.010	36±6	4.56±0.05
3.3	0.964±0.005	26±4	4.53±0.02
4.5	0.962±0.016	23±3	4.52±0.07
5.8	0.970±0.019	20±4	4.56±0.09
7.0	0.966±0.021	17±2	4.54±0.10
8.3	0.966±0.011	15±2	4.54±0.05
12.0	0.964±0.034	11±1	4.54±0.16
			Mean 4.54±0.01‡

* Although the volume indicator reports only two decimal places, the third has been calculated from the mean.
† Excluding the end-point delay time (20 s) (see text).
‡ Mean and standard deviation (σ).

Table 2 shows that the precision is good even for difficult solid samples of aminobenzylpenicillin.

Determination of water in penicillins

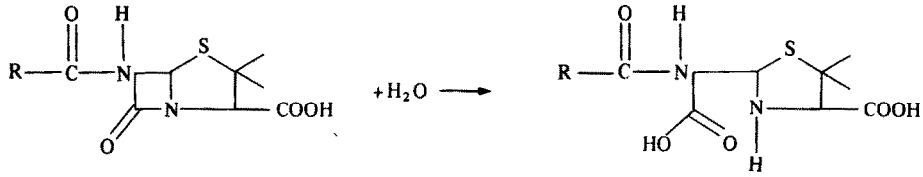
When titrating samples which give 'creeping end-points', iodine-consuming side reactions have to be considered. However, the difficulty in finding the true content of water can be considerable. One example concerns aminobenzylpenicillin trihydrate, which by KF-titration gives higher values than those calculated, and lower values than those by loss on drying. Some of the reasons for this might be:

1. The penicillin always contains some penicilloic acid, which at least in aqueous solution consumes iodine (iodometric titration is in fact a standard method for the assay of penicillins after hydrolysis to penicilloic acid).
2. On drying (at, for example, 60°C) there is always some decomposition of the penicillin to penicilloic acid with a corresponding uptake of water. This has to be considered when using other methods where heating is applied (i.e. gas chromatography).

Table 2
Titration of water in aminobenzylpenicillin. Speed of motor-driven burette: 2–3 ml/min; reagent: Hydranal titrant and solvent

Sample batch	Sample weight (mg)	Titrant (ml)	Water content* (%)
1	113.4	3.15	13.05
	127.1	3.49	12.91
2	106.4	2.90	12.81
	117.4	3.22	12.89
3	118.2	3.32	13.20
	108.5	3.03	13.13
	115.3	3.24	13.21
4	104.9	2.92	13.08
	118.4	3.28	13.02

* Standard deviation: $\sigma = \pm 0.027$.
Relative standard deviation: $S_{rel} = 0.2\%$.



Scheme 1

If the content of water in a penicillin is determined with a fast reagent (i.e. KF Rapid), a lower value is often obtained as compared to a standard reagent (KF standard). When this difference in water content (ΔH_2O) is calculated as penicilloic acid with the assumption that one mole consumes 8 equivalents of iodine (which is the case in aqueous solution), the result follows the mercurimetrically-titrated content of penicilloic acid (PCA) in the case of aminobenzylpenicillin (Table 3).

Table 3
Determination of water and penicilloic acid (PCA) in aminobenzylpenicillin trihydrate

Sample	Loss on drying (%)	H_2O content KF Standard (%)	H_2O content KF Rapid (%)	ΔH_2O (%)	PCA (from Δ) (%)	PCA* (%)
1	13.35	13.70	13.35	0.35	1.79	1.81
3	13.09	13.52	13.38	0.14	0.71	0.81
4	13.16	13.55	13.43	0.12	0.61	0.48

* By mercurimetric titration.

Similar titrimetric determinations of water in carboxybenzylpenicillin (stored samples) did not give the same agreement for the penicilloic acid values. However, a plot of the content of penicilloic acid, calculated from $6.1\Delta H_2O$,* against that determined

*The factor 6.1 is calculated from the ratio of molecular weights of penicilloic acid and water, divided by their iodine equivalent.

mercurimetrically, gives a straight line ($y = 0.94x - 3.95$), with a correlation coefficient of 0.93 (Fig. 3). This indicates that at $\triangle H_2O = 0$ about 4.2% of penicilloic acid is still present. One explanation for this is that some other impurity, that does not consume iodine, or that consumes both KF reagents, is co-titrated mercurimetrically and measured as penicilloic acid.

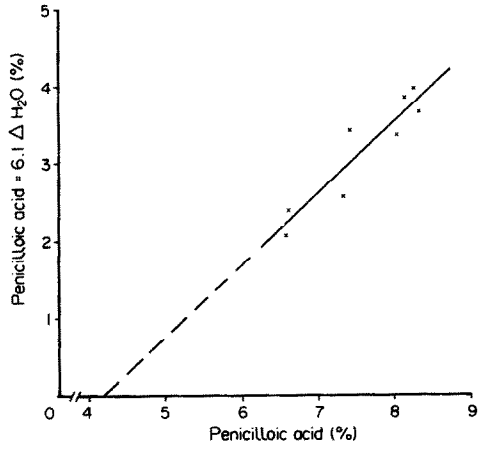


Figure 3
The difference in water content ($\triangle H_2O$) for carboxybenzylpenicillin (obtained when using conventional and fast KF reagents), calculated as penicilloic acid, plotted against mercurimetrically determined values of penicilloic acid.

In bacampicillin (1′-ethoxycarbonyloxyethyl-ester of ampicillin) containing 2–6% of penicilloic acid, the corresponding value calculated from $\triangle H_2O$ was 0.2–1.2% when Hydranal was used. In tablets with 1.5 ± 0.2% penicilloic acid, the corresponding value was 0.66 ± 0.15% when KF Rapid was used. In very degraded tablets containing 17% penicilloic acid, no significant value for $\triangle H_2O$ was obtained. The interpretation of this is complicated by the presence of the ester group. Ampicillin, for example, is attacked by bromine during bromometric titration, while the esterified ampicillin (bacampicillin) is not. The reaction between penicilloate and iodine is also probably affected by the ester group.

In cloxacillin monohydrate containing 0.92 ± 0.09% penicilloic acid, values of 3.89 ± 0.03% and 3.89 ± 0.05% of water ($n = 12$) were measured using conventional KF reagent and KF Rapid respectively, so that $\triangle H_2O = 0$. However, the theoretical water content (monohydrate = 3.78%) is 0.11% lower, which would be equivalent to 0.73% of penicilloic acid, i.e. a value near the mercurimetrically determined value. If this is not simply coincidental, both reagents would co-titrate what is in this case the more reactive penicilloic acid.

When Hydranal was used as a fast-reacting reagent instead of KF Rapid, about the same $\triangle H_2O$-values were measured on samples of ampicillin trihydrate, as shown in Table 3. But the sodium salt of ampicillin gave $\triangle H_2O$-values near zero, in spite of the high content of penicilloic acid (2–5%).

The reason for this is the low buffer capacity of Hydranal solvent. Addition of 200 mg of the sodium salt resulted in an increase of pH* from 6.0 to 7.1, which is too high for optimal reaction rate. This was shown when Hydranal solvent was titrated with methanolic sulphuric acid and potassium hydroxide (Fig. 4).

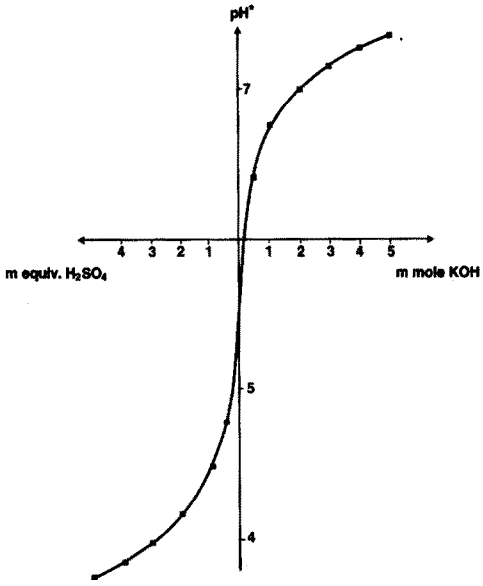


Figure 4
20 ml of Hydranal solvent titrated with methanolic H_2SO_4 (2.5 M) and KOH (5 M).

Addition of 50 µl of conc. sulphuric acid (before the pre-titration) to compensate for the change in pH* gave sharper end-points and results which were practically independent of the speed of titration. Table 4 shows samples containing 3.4% penicilloic acid, which is equivalent to $\triangle H_2O$=0.67%. As indicated in the table, this is readily obtained even when using a fast-reacting reagent (instead of the conventional one), but outside the optimum range of pH*. The true content of water in this sample was not more than 0.2%.

Table 4
Titration of water in ampicillin sodium with and without adjustment of pH* by sulphuric acid. Hydranal titrant and solvent were used

Speed of tritration	H_2SO_4 added (µl)	Sample (mg)	Titrant (ml)	H_2O (%)
Normal	0	183.5	0.15	0.38
	0	206.7	0.17	0.39
Slow	0	221.3	0.40	0.85
	0	195.5	0.28	0.67
Normal	50	226.7	0.09	0.19
	50	245.4	0.09	0.17
Slow	50	210.4	0.10	0.22
	50	210.0	0.09	0.20

Blank values

Relevant blanks for samples containing iodine-consuming substances can in some cases be obtained by using KF solvents where sulphur dioxide is excluded and replaced by, for example, acetic or methanolic sulphuric acid to restore the original pH*-value.

When such blanks were titrated on samples of ampicillin sodium dissolved in methanol containing 1 M acetic acid and 2 M pyridine (pH* = 5.5), the measured iodine

consumption corresponded to values for the water content up to 60% of that obtained by normal KF titration, using conventional pyridine-based reagents. This is in good agreement with earlier results. However, blanks might give misleading results. Samples containing sulphite are an obvious example, and in many other reactions with iodine the presence of water is necessary.

Another misleading example was 6-APA (6-aminopenicillanic acid) containing 6.2% of impurities as measured mercurimetrically. By using conventional KF reagent, 4.0% of water was reported, while 0.0% was reported using Hydranal. The blanks were 0.0% in 1 M methanolic H_2SO_4 containing pyridine or diethanolamine (pH* 4 and 5.9 respectively).

In the pyridine-based blank solutions, aminobenzylpenicilloic acid gave a creeping end-point and consumed more iodine than in the diethanolamine-based blank (*ca* 5.4 and 3.8 equivalents/mole of penicilloic acid, respectively).

Conclusions

Fast reacting Karl Fischer reagents, now commercially available, have great advantages as compared to conventional pyridine-based reagents, especially with respect to the stability of the titration end-point. This, together with the instrumental optimization proposed, makes it possible to radically reduce the time of titration, thereby also improving the selectivity of analysis. The instrument has been in daily routine use since the end of 1981 without any trouble and with unchanged precision. Penicilloic acid is to a great extent co-tritated during KF titration for determination of water in penicillins when using slow-reacting reagents. This error can in many cases be reduced by using fast methods.

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