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The interference of penicilloic acids with Karl Fischer titration of penicillins

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Abstract

Phenoxymethylpenicilloic acid monohydrate was titrated with 12 different brands of Karl Fischer reagent. It was shown that only the four pyridine-containing reagents gave correct results for water content, providing that the sample solution was cooled in an ice bath or that the delay time of the amperometric end-point determination was 10 s instead of 30 s, as prescribed by the European Pharmacopoeia. The other reagents always gave results, which were too high. When these reagents were used to titrate the water in sodium amoxicillin, sodium carbenicillin or sodium ticarcillin the same conclusions could be drawn with regard to the accuracy of the results, the usefulness of the reagents and the reaction conditions. These penicillins may contain up to 9% penicilloic acids. The maximum relative error obtained with pyridine-free reagents was about 50% for ticarcillin. It has been confirmed that penicilloic acids interfere with Karl Fischer titration of water and that this interference is greatly dependent on the brand of reagent and on the reaction conditions.

Keywords: Interference; Karl Fischer titration; Penicillins; Penicilloic acids

1. Introduction

Interference of degradation products (i.e. penicilloic acids) with semi-microdetermination (Karl Fischer titration) of water in penicillins was mentioned previously by Lindquist [1]. Penicilloic acids are oxidized by iodine and in fact iodimetric titration is a standard method for the determination of penicillins, which first have to be converted into penicilloic acids by hydrolysis. The reaction is rather slow and is pH-dependent. Interference with Karl Fischer titration was reported to decrease on increasing the titration speed through the use of fast reagents and a home-built electronic control unit. In these experiments penicillins were used which contained relatively small amounts of penicilloic acids [1].

The phenomenon is now studied using pure phenoxymethylpenicilloic acid, containing about 5% water. The influence of the type of Karl Fischer reagent has been examined by using

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reagents of different brands: one-component reagents as well as two-component reagents. The influence of the speed of the titration has been examined by adapting the delay time at the end of the titration. This is the time during which the deflection of the microammeter should persist, before the titration is ended. The influence of cooling the reacton vessel has also been examined as well as the influence of purging the reaction vessel with dry nitrogen. Titrations are also carried out using penicillins which contain relatively large amounts of penicilloic acids such as sodium amoxicillin, disodium carbenicillin and disodium ticarcillin.

2. Experimental

2.1. Solvents and chemicals

Methanol (HPLC grade) was obtained from Rathburn (Walkerburn, UK); pyridine, iodine and methoxyethanol were from Acros Chimica (Beerse, Belgium); sodium tartrate dihydrate (reference substance for Karl Fischer titration) was a p.a. reagent from E. Merck (Darmstadt, Germany) and sulphur dioxide was from Matheson Union Carbide (Oevel, Belgium). Water was doubly-distilled and boiled prior to use. Disodium ticarcillin, disoium carbenicillin and sodium amoxicillin samples were obtained from Beecham (Heppignies, Belgium). Phenoxymethylpenicilloic acid was prepared in the laboratory according to Munro et al. [2].

2.2. Apparatus

A Karl Fischer Automat 633 combined with a Multi-Dosimat 645 and a Multi-Bürette E485 (Metrohm, Switzerland) were used. The titration speed 5 ml min^{-1}.

A gas chromatograph DN 200 with a series 11 thermal conductivity detector (Delsi-Nermag, Argenteuil, France) and a 5 ft × 1/8 in Porapak column (Alltech, Laarne, Belgium) was used. The temperature of the column was maintained at 130°C. Nitrogen was used as the carrier gas, methanol as the internal standard and 2-propanol as the solvent.

2.3. Karl Fischer reagents

Several batches of Karl Fischer reagents were prepared according to the procedure described in the European Pharmacopoeia (Ph. Eur.) [3]. Other Karl Fischer reagents were obtained from J.T. Baker (Deventer, The Netherlands); BDH (Poole, UK); E. Merck and Riedel de Haën (Seelze, Germany).

2.4. Procedure

The method prescribed in the Ph. Eur. was followed [4]. First, the solvent was titrated to the end-point. The sample was introduced into the titration vessel and titrated. For phenoxymethylpenicilloic acid 0.100 g was used; for the penicillins the prescribed amounts were used: 0.150 g for disodium ticarcillin [5] and disodium carbenicillin [6], 0.400 g for sodium amoxicillin [7]. The Ph. Eur. prescribes that at the end of the titration the deflection of the microammeter should persist for not less than 30 s (delay time). Titrations were stopped manually when the total titration time was more than 20 min. Independent titrations were performed, the vessel being emptied after each titration and fresh solvent added. Most titrations were performed while purging the reaction vessel with dry nitrogen. Standardisation was performed daily with sodium tartrate dihydrate, except for J.T. Baker modified solvent (8840) where a standard containing water was used, because the tartrate was not soluble.

3. Results and discussion

The original Karl Fischer (KF) reagent contains iodine, sulphur dioxide, pyridine and methanol [8]. Pyridine is present to buffer the pH and methanol ensures the stoichiometry of the reaction by forming methylsulphite with sulphur dioxide, which is then oxidised by iodine into methylsulphate with consumption of one equivalent of water. Methylsulphate cannot react further with water whereas, in the absence of methanol, sulphur trioxide is formed, which can further

react with water, leading to an undefined stoichiometry. Excess iodine is detected amperometrically. Several KF reagents with different compositions are now available, as one-component or two-component reagents. A one-component reagent is a titrant containing iodine, sulphur dioxide and a suitable base or buffer in methanol or methoxyethanol. A two-component reagent is a system where the sample is dissolved in the solvent provided by the manufacturer. This solvent usually contains sulphur dioxide and a base dissolved in methanol or methoxyethanol. The titrant contains iodine in methanol or methoxyethanol. Other components may be present. More details concerning the composition of the Karl Fischer reagents used in this study have been published elsewhere [9]. The Ph. Eur. iodosulphurous reagent is a classical pyridine-containing reagent.

Penicillins always contain at least some penicilloic acid, which is formed by hydrolysis of the β-lactam ring. In order to study the interference of penicilloic acids in KF titration of penicillins, experiments were first carried out with pure penicilloic acid, where interference would be easier to detect. Phenoxymethylpenicilloic acid, derived from phenoxymethylpenicillin or penicillin V, was used for this purpose. When phenoxymethylpenicilloic acid is prepared by alkaline hydrolysis in aqueous medium and acidificaiton, it crystallizes as the monohydrate [2]. This corresponds to a water content of about 5%. The exact amount of water in penicilloic acids cannot be determined by loss on drying because they are liable to decompose by decarboxylation during heating. Therefore, as an alternative method for KF titration, the water content was determined by gas chromatography. The result was 5.1%.

Results obained by KF titration of phenoxymethylpenicilloic acid are shown in Table 1. In all, 12 different KF reagents were used: classical one-component reagents with pyridine, one-component reagents without methanol, one-component reagents without pyridine and two-component reagents. Two titration delay times were used; that prescribed by the Ph. Eur. (30 s) and a shorter delay time of 10 s. The titrations were either carried out at room temperature or with cooling in an ice bath. Most experiments were carried out in a flow of dry nitrogen. The results mention the mean (% m/m water) of several titrations. The numbers of titrations and the realtive standard deviations (RSDs) are mentioned in parentheses. The maximum total titration time (T) is also reported.

The results obtained with a delay time of 30 s show that at room temperature the values are always high. The pyridine-free one-component reagents and the two-component reagents show the greatest differences from the value determined by gas chromatography (5.1%). The deviations most often correspond to more than 100%. When cooling in an ice bath the pyridine-containing one-component reagents (1–4) give a value slightly above 5.1%. The other reagents are not greately influenced by the temperature. At the same time it is observed that, for the pyridine-containing reagents, the total reaction time is greatly reduced. Apparently the side-reaction with penicilloic acid is too slow in pyridine and at 0°C to cause a measurable problem. For the other reagents the cooling is not sufficient to exclude the side-reaction. In the experiments under nitrogen it is shown that the results obtained with reagents 1–4 are very close to 5.1%. The nitrogen flow excludes the influence of atmospheric moisture.

The results with a delay time of 10 s were always obtained in a nitrogen environment. Reduction of the delay time allows one to reduce the total reaction time. Shorter reacton times were previously advocated by Kågevall et al. [10] when performing flow-injection Karl Fischer analysis of penicillins. It is now observed that cooling is not necessary to obtain good results with pyridine-containing reagents. One pyridine-free reagent (5) also gives reasonable results. All other results differ by more than 100% from the expected value (5.1%). It is observed that reaction speed alone is not sufficient to guarantee correct results. Most two-component reagents are also very fast, but apparently the composition of these reagents always allows side-reactions to occur. Some experiments with two-component reagents could not be carried out because the sample was not soluble in 30 ml of the solvent.

Table 1
Karl Fischer titrations of phenoxymethylpenicilloic acid (% m/m water)[a]

KF reagent	Normal titration delay time (30 s)			Shortened titration delay time (10 s)	
	Room temp.	Ice bath	Ice bath (nitrogen)	Room temp. (nitrogen)	Ice bath (nitrogen)
One-component reagents					
1. Ph. Eur. iodosulphurous	8.69 (3,13.8) $T=10$	5.30 (3,1.6) $T=5$	5.08 (7,3.3) $T=5$	5.08 (3,0.7) $T=1$	5.17 (4,4.4) $T=1$
2. Merck titrant A+B (9246+9247)	10.01 (3,8.4) $T=20$	5.24 (5,0.9) $T=4$	5.20 (5,1.3) $T=5$	5.19 (2,4.1) $T=1$	5.33 (2,2.4) $T=1.5$
3. Merck methanol-free (9248)	7.86 (7,11.9) $T=24$	5.26 (6,2.0) $T=8$	5.13 (7,2.3) $T=3$	5.08 (3,6.7) $T=1.5$	5.09 (4,4.9) $T=1.5$
4. Merck rapid methanol-free (9245)	9.47 (5,5.5) $T=10$	5.43 (4,0.2) $T=4$	5.14 (4,5.7) $T=4$	5.66 (3,0.7) $T=1$	5.12 (4,4.1) $T=1$
5. Merck pyridine-free (9258)	13.79 (3,1.7) $T=10$	8.66 (3,16.3) $T=12$	NP	5.30 (4,0.7) $T=1$	5.35 (4,0.7) $T=1$
6. Riedel de Haën hydranal composite pyridine-free (34805)	11.16 (3,0.6) $T=4$	11.67 (3,1.6) $T=9$	NP	11.41 (3,2.8) $T=3$	11.60 (4,2.3) $T=3$
Two-component reagents					
7. Merck titrant U(9233) solvent K(9221)	13.99 (3,5.3) $T=20$	INS	NP	11.61 (2,2.3) $T=10$	INS
8. Merck titrant U(9233) solvent (9241)	12.89 (2,1.7) $T=20$	13.20 (3,2.9) $T=25$	NP	13.34 (3,3.2) $T=1$	12.62 (4,4.4) $T=1$
9. Riedel de Haën hydranal titrant (34801) solvent (34800)	12.04 (3,0.7) $T=9$	11.99 (3,1.9) $T=5$	NP	13.87 (3,2.5) $T=1$	13.53 (4,1.7) $T=1$
10. BDH titrant (19265) solvent (19266)	12.40 (4,2.7) $T=9$	12.79 (4,4.2) $T=17$	NP	13.04 (3,3.0) $T=1$	13.53 (4,3.3) $T=1$
11. J.T. Baker titrant (8842) solvent (8855) sprint	12.38 (4,6.6) $T=3$	13.07 (4,4.6) $T=16$	NP	11.07 (3,2.7) $T=1$	11.05 (3,2.5) $T=2$
12. J.T. Baker titrant (8842) solvent (8840) modified	12.69 (3,7.1) $T=8$	13.47 (3,0.3) $T=12$	NP	11.31 (3,2.1) $T=1$	11.95 (4,3.8) $T=1$

[a]The number of titrations (n) and the RSD are given in parentheses (n, RSD). INS = insoluble under the conditions used. NP = not performed. T = maximum titration time observed (min). Aliquots of 100 mg and 30 ml portions of solvent were used.

The degradation products of the samples of sodium amoxicillin, disodium carbenicillin and disodium ticarcillin were found to comply with the prescriptions of the corresponding Ph. Eur. monograph [5–7]. The degradation products are a measure of the hydrolysed β-lactams which may

Table 2
Karl Fischer titrations of commercial penicillins (% m/m water)[a]

KF reagent	Amoxicillin sodium		Carbenicillin sodium		Ticarcillin sodium	
	A	B	A	B	A	B
One-component reagents						
1. Ph. Eur. iodosulphurous reagent	3.95 (5,1.6) $T=9$	3.32 (5,3.5) $T=6$	4.34 (5,1.7) $T=10$	4.00 (5,2.3) $T=6$	4.71 (5,1.7) $T=10$	4.26 (5,3.2) $T=6$
2. Merck titrant A + B (9246 + 9247)	NP	3.24 (4,2.5) $T=8$	NP	4.13 (4,3.4) $T=6$	NP	4.23 (3,6.8) $T=6$
3. Merck methanol-free (9248)	3.89 (4,4.3) $T=7$	3.47 (4,3.1) $T=4$	4.71 (4,3.3) $T=8$	4.19 (4,2.5) $T=5$	5.17 (6,1.8) $T=7$	4.55 (4,6.8) $T=5$
4. Merck rapid methanol-free (9245)	3.75 (4,2.1) $T=3$	3.34 (3,9.4) $T=3$	4.64 (3,3.7) $T=5$	4.14 (4,8.7) $T=3$	4.96 (3,3.3) $T=7$	4.39 (4,8.4) $T=5$
5. Merck pyridine-free (9258)	NP	4.16 (4,8.2) $T=3$	NP	4.63 (4,4.6) $T=3$	NP	5.76 (6,3.8) $T=7$
6. Riedel de Haën hydranal composite pyridine-free (34805)	NP	3.54 (3,5.9) $T=3$	NP	4.40 (3,7.8) $T=2$	NP	5.78 (3,8.8) $T=5$
Two-component reagents						
7. Merck titrant U(9233) solvent K(9221)	NP	INS	NP	INS	NP	INS
8. Merck titrant U(9233) solvent (9241)	NP	INS	NP	INS	NP	INS
9. Riedel de Haën titrant (34801) solvent (34800)	NP	INS	NP	INS	NP	INS
10. BDH titrant (19265) solvent (19266)	NP	INS	NP	4.73 (2,1.8) $T=2$	NP	INS
11. J.T. Baker titrant (8842) solvent (8855)	NP	3.63 (4,3.1) $T=5$	NP	5.67 (4,8.2) $T=18$	NP	6.40 (4,7.0) $T=20$

[a] A: titrations performed at room temperature and under nitrogen. B: titrations performed at 0°C and under nitrogen. The number of titrations (n) and the RSD are given in parentheses (n, RSD). INS = insoluble under the conditions used. NP = not performed. T = maximum titration time observed (min). Sample size was 400 mg for sodium amoxicillin and 150 mg for disodium carbenicillin or disodium ticarcillin. Aliquots of 30 ml of solvent were used.

react with iodine. The amounts were 9% in sodium amoxicillin, 7% in disodium carbenicillin and 6% in disodium ticarcillin. The results of KF titration of the penicillins are shown in Table 2. All the experiments were carried out with a delay time of 30 s, under nitrogen. The only variables were the brand of KF reagent and the reaction temperature. A shorter delay time was not used due to lack of sample and also because it was not considered to be necessary: the effect of a shorter delay time is adequately shown in Table 1. With the penicillins there was also a difference between titration at room temperature and at 0°C, the latter giving lower results and shorter titration

times. At 0°C the pyridine-containing reagents (1–4) gave similar results and, considering the behaviour observed for phenoxymethylpenicilloic acid, they were accepted as the correct values. For reagents 1–4 the maximum relative difference with the result obtained at room temperature was 18% for amoxicillin, 14% for carbenicillin and 19% for ticarcillin. The pyridine-free one-component reagents (5,6) gave higher values, as expected. Most of the two-component reagents could not be used because the samples were not soluble in 30 ml of the solvent. As expected, two-component reagents always gave values which were too high. The maximum relative difference with the result obtained at 0°C with reagents 1–4 was 24% for amoxicillin, 38% for carbenicillin and 47% for ticarcillin. The observed differences are less important than for the titrations in Table 1 because the penicillin samples only contain 6–9% of penicilloic acids.

4. Conclusion

It has been shown that the presence of penicilloic acids may significantly influence the Karl Fischer titration. Much depends upon the choice of the reagents. So-called fast reagents do not always give reliable results. Pyridine-containing one-component reagents give the best results but even they lead to overestimation when Ph. Eur. conditions are used. Correct values are obtained with the pyridine-containing one-component reagents when the sample solution is cooled in an ice bath or the delay time is reduced from 30 s to 10 s. Purging the reaction vessel with dry nitrogen slightly improves the result. This paper shows clearly that the Karl Fischer reagent officially prescribed by the Ph. Eur. should not be replaced by other reagents without rigorous validation.

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