Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 5, pp. 461–472, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

The determination of water in erythromycin by Karl Fischer titration*

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Abstract: Important interlaboratory variation was obtained for the water content of erythromycin samples as determined by the Karl Fischer method. It is demonstrated that the variation is related to the type of reagents used. In poorly buffered systems erythromycin enol ether and water are formed by acid degradation of erythromycin. However, when an appropriate solvent is used, accurate titration of water in erythromycin is possible. A 10% m/v solution of imidazole in methanol is preferred to pyridine or a mixture of pyridine and methanol for it has a good buffer capacity, it lacks the unpleasant odour of pyridine and it allows a high titration speed.

Keywords: *Erythromycin; determination of water; Karl Fischer titration.*

Introduction

Important interlaboratory variation was obtained for the water content of erythromycin samples as determined by the Karl Fischer (KF) method [1]. This method is prescribed by several official texts for erythromycin and erythromycin esters [2, 3].

The structures of erythromycin A (EA) and of the corresponding esters are shown in Fig. 1. Since KF titrations are mainly performed in alcoholic media, the carbonyl at C9 can be expected to interact with alcohol. Formation of ketals by reaction of ketones and methanol or other alcohols is a well-known problem in KF titration [4]. The water evolved by ketal formation gives "creeping end-points" and erratic results. The phenomenon has been reported for small chain ketones and is less likely to occur with erythromycin. It is also known that EA is transformed into erythromycin A enol ether (EAEN) by acid catalysed dehydration [5] (see Fig. 1). The acid catalysis and therefore the speed of degradation may be dependent upon such factors as the composition of the KF reagent and the solvent used which may be the reason for the interlaboratory variability.

Several KF reagents with different compositions are now available. The original reagent contained iodine, sulphur dioxide, pyridine and methanol. Peters and Jungnickel proposed the substitution of methanol by methoxyethanol to improve the stability of the reagent [6]. No major modification was proposed until Verhoef and Barendrecht pointed

*Dedicated to Professor H. Vanderhaeghe on the occasion of his 65th birthday.

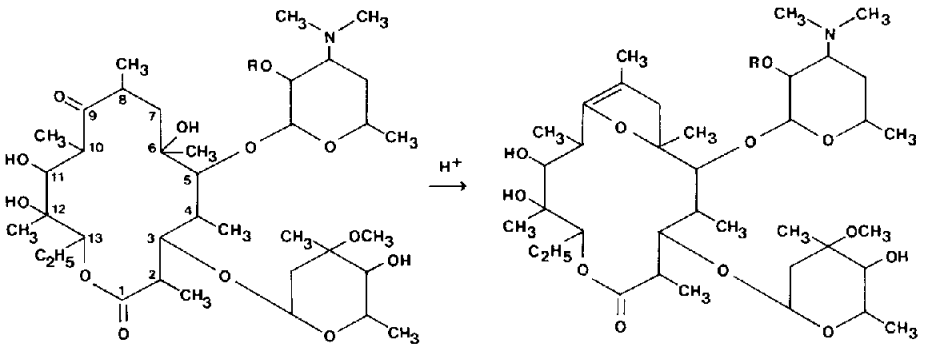

Figure 1
Degradation of erythromycin to erythromycin enol ether.

	R
Erythromycin A (EA)	H
Erythromycin A stearate	H .$C_{18}H_{36}O_2$
Erythromycin A propionate	$COCH_2CH_3$
Erythromycin A estolate	$COCH_2CH_3.C_{12}H_{25}OSO_3H$
Erthromycin A ethylsuccinate	$COCH_2CH_2COOC_2H_5$.

out that pyridine is not an essential component of the reagent and only acts as a buffer [7]. This gave the incentive to use bases other than pyridine.

The aim of this study was to investigate the problems associated with the KF titration of erythromycin and to determine conditions allowing accurate titration.

Experimental

Solvents and chemicals

Water was distilled twice and boiled prior to use. Methanol (HPLC grade) was obtained from Rathburn (Walkerburn, Scotland); pyridine p.a. from U.C.B. (Brussels, Belgium); chloroform and dichloromethane from Janssen Chimica (Beerse, Belgium); carbon tetrachloride from Belgolabo (Overijse, Belgium); imidazole, ammonia 25% and sodium tartrate dihydrate (reference substance for KF) were p.a. reagents from E. Merck (Darmstadt, FRG). Ready-made silica gel thin layer plates (E. Merck) were used.

Erythromycin was obtained from Cepa (Madrid, Spain), erythromycin stearate from Proter (Milan, Italy), erythromycin propionate from Roussel Uclaf (Romainville, France), erythromycin estolate (erythromycin propionate laurylsulphate) from Eli Lilly (Giessen, FRG) and erythromycin ethylsuccinate from Abbott (St. Remy s/Arre, France). Reference samples of EA and EAEN were prepared in the laboratory [8].

Apparatus

A Karl Fischer Automat 633 combined with a Multi-Dosimat 645 and a Multi-bürette E485 (Metrohm, Herisau, Switzerland). Titration speed: 4 ml min^{-1}.

Apparent pH (pH*) measurements were determined with a Beckman pH meter (Fullerton, CA, USA). The combined glass electrode was calibrated with aqueous buffer solutions. Measurements were performed on 2 ml samples from the titration vessel, each diluted with 2 ml of water. These experiments were carried out during titrations in the absence of erythromycin, using dilutions of water in methanol.

Karl Fischer reagents

Several batches of KF reagents were prepared according to the procedures described in the European Pharmacopoeia (Ph. Eur.) [2]. The other reagents were obtained from J.T. Baker, Deventer, the Netherlands; British Drug House (BDH) (Poole, UK); E. Merck; May and Baker (Dagenham, UK) and Riedel de Haën (Seelze, FRG). Table 1 gives a survey of the components of the different KF reagents known so far. The quantitative compositions of the commercial KF reagents are not available. A one component reagent is a titrant containing iodine, sulphur dioxide and a base in methanol or methoxyethanol. In a two component reagent system the sample is dissolved in the solvent provided by the manufacturer. This contains sulphur dioxide and a base in methanol or methoxyethanol. The titrant contains iodine in methanol or methoxyethanol, and other components may also be present.

Determination of water, procedure

The method prescribed by the Ph. Eur. was followed. Instead of 20 ml, 25 ml of solvent was used. First the solvent was titrated to the electrometric end-point. The delay time for the end-point was set at 30 s. The prescribed amount of erythromycin (0.200 g) was introduced into the titration vessel, the solution was stirred for 1 min and titrated.

Generally, titrations were stopped manually when the time exceeded 15 min. Independent and consecutive determinations were carried out. For independent determinations the titration vessel was emptied after each titration and fresh solvent added. For consecutive determinations the titration vessel was not emptied between titrations. Standardisation of the reagents was performed with sodium tartrate dihydrate. In a number of experiments this standardisation was checked with a dilution of water in methanol, prepared to contain about 3 mg ml^{-1}. Titrations were performed on 5.0 ml aliquots. Results were corrected for the water content (about 0.015% m/v) of the methanol. Reagents were standardised at least daily.

Thin layer chromatography

Thin layer chromatography (TLC) was used to follow the fate of erythromycin during the titration. The TLC method has been discussed previously [9, 10]. Titrations were performed with the different titrants and with methanol as the solvent, or with the solvent provided by the manufacturer. Samples (5 µl) were taken at 0, 5, 10, 15 and 20 min after the start of the titration notwithstanding the fact that the titration might have been completed meanwhile. The samples were applied directly to the plate. The mobile phase was dichloromethane–methanol–25% ammonia (90:9:1.5). EA and EAEN were applied as reference materials.

Gas chromatography

Head-space gas chromatography was used to investigate the presence of solvents in the erythromycin sample. A Pye 105 chromatograph (Cambridge, UK) equipped with a flame ionisation detector was used with a Porapak Q (Chrompack, Middelburg, the Netherlands) column (¼ inch, 5 ft). Flow rates were 15 ml min^{-1} for hydrogen and nitrogen and 300 ml min^{-1} for oxygen. Oven temperature was 175°C. A 1.0 g sample of erythromycin was suspended in 5 ml of freshly distilled water in a 10 ml vial. The capped vial was heated for 15 min in a boiling water bath. A 0.5 ml head-space sample was injected in the chromatograph.

Table 1
Qualitative composition of the different Karl Fischer reagents

Reagents	Chemicals present							
	Iodine	Sulphur dioxide	Pyridine	Imidazole	Diethanolamine	Sodium acetate	Methanol	Methoxyethanol
One component								
Ph. Eur. "iodosulphurous reagent"	x	x	x					x
BDH								
Solution A (19237)		x	x				x	
Solution B (19238)	x						x	
Merck								
"Rapid" methanol-free (9245)	x	x	x					x
Methanolfree (9248)	x	x	x					x
May and Baker (L327)	x	x	x					x
Riedel de Haën Hydranal composite 5 (34805)	x	x		x				x
Two component								
J.T. Baker Reaquant								
titrant (8842)	x						x	
solvent "modified" (8840)		x				x	x	
solvent "sprint" (8885)		x			x		x	
BDH								
titrant (19265)	x	x						x
solvent (19266)		x					x	
Riedel de Haën Hydranal								
titrant (34801)	x						x	
solvent (34800)		x			x		x	
Merck								
titrant (9243)	x						x	
titrant U (9233)	x	x						x
solvent (9241)		x					x	
solvent K (9221)		x						x

Results

Results for the KF determination of erythromycin are summarised in Table 2 for the one component reagents and in Table 3 for the two component reagents. The results should be compared with the loss on drying (LOD). The LOD of seven 1.000 g aliquots of erythromycin was determined by heating at 60°C under reduced pressure (<0.1 kPa) till constant mass (3 h). Mean: 4.48% m/m, R.S.D. (Relative Standard Deviation): 3.4%.

The possible influence of organic solvents on the LOD was checked by gas chromatography under conditions where e.g. acetone may be detected at the 0.05% m/m level and *n*-butanol, the least volatile reference compound used, at the 0.1% m/m level. No solvents were detected.

Titration time ranges in min (TT) are also mentioned in Tables 2 and 3. Titrations were usually stopped manually when TT exceeded 15 min. This is indicated in the tables by "manual stop". Thus the real TT can even exceed 15 min.

With one component reagents (Table 2) three different solvents were used. Methanol is not only the solvent prescribed by the Ph. Eur., but probably also the solvent most frequently used in KF titrations of pharmaceuticals. For the determination of water in ketones the use of pyridine is often suggested in order to avoid ketal formation by methanol [4]. Chloroform–carbon tetrachloride–methanol (2:2:1) is the solvent prescribed by the USP XXI [3]. With some combinations of reagent and solvent, the titrations were repeated on different days to determine day-to-day variations. The groups of results are reported separately in the Tables. It is observed in Table 2 that when pyridine was used as the solvent, values obtained in independent or consecutive titrations were always close to those obtained by LOD. With methanol as the solvent in independent titrations, higher values were obtained. Consecutive titrations in methanol, which were performed only with reagents D and F, showed higher figures as compared to the independent titrations. The solvent prescribed by the USP gave results comparable with those obtained with methanol. For all the reagents TLC revealed the formation of EAEN during titration with methanol as the solvent. EAEN is formed from EA by acid catalysis and with loss of one equivalent of water (see Fig. 1). The presence of EAEN was also confirmed by HPLC. The same HPLC method was used previously for the separation of erythromycin derivatives [11, 12]. Details on this HPLC method are discussed elsewhere [8].

Independent determinations with two component reagents (Table 3) are also in close agreement with LOD. When performing consecutive determinations, the result increased after a certain number of determinations had been carried out. Except for reagents I and L, this occurred after the second or third titration. Here TLC also revealed the formation of EAEN. Table 4 presents the results obtained for the esters of erythromycin and for erythromycin stearate as determined with the Ph. Eur. iodo-sulphurous reagent in three different solvents [methanol, pyridine and pyridine: methanol (1:1)]. With methanol as the solvent higher values were found. Consecutive titrations in pyridine:methanol (1:1) give results comparable with those obtained with pyridine.

Discussion

It is clear that the variability of the results found is related to the type of reagents used. From the results of Table 2 it may be concluded that the choice of the solvent is most important and that methanol is not a good solvent for KF titrations of erythromycin.

Table 2
Water content (% m/m) in an erythromycin sample as obtained with one component Karl Fischer reagents

KF reagent	Solvent: Methanol		Pyridine		CCl_4-$CHCl_3$-CH_3OH (2:2:1) (USP)	
	Independent	Consecutive	Independent	Consecutive	Independent	Consecutive
A						
Ph. Eur.	5.60 (3,6.0)	NP	4.56 (3,4.7)	4.64 (5,1.7)	5.79 (3,4.3)	NP
"iodosulphurous reagent"	TT = 8 → 15		TT = 3 → 7	TT = 1 → 2	TT = 10 → 15	
	5.83 (3,9.5)		4.65 (3,2.3)	4.49 (4,1.0)	5.51 (3,5.6)	
	TT = 10 → 11		TT = 4 → 9	TT = 2 → 4	TT = 6 → 7	
			4.49 (3,0.5)	4.53 (8,1.4)		
			TT = 4 → 5	TT = 2 → 4		
B						
BDH	6.40 (3, 1.7)	NP	4.60 (3,4.8)	4.60 (4,1.1)	6.54 (3,1.3)	NP
standard KF	TT = 22 → 24		TT = 2 → 5	TT = 2 → 6	TT = 19 → 25	
(19237) + (19238)				4.62 (4,3.0)		
				TT = 2 → 5		
C						
Merck "Rapid"	5.42 (3,9.0)	NP	4.45 (3,1.0)	4.45 (3,1.0)	4.72 (3,8.0)	NP
Methanol-free (9245)	TT = 1 → 15 (MS)		TT = 4 → 6	TT = 2 → 3	TT = 1 → 10	
D						
Merck	5.00 (6,1.4)	6.08 (5,11.0)	4.71 (5,0.5)	4.66 (5,0.5)	NP	NP
Methanol-free (9248)	TT = 4 → 5	TT = 3 → 7	TT = 4 → 7	TT = 2 → 6		
	4.89 (5,2.0)	5.25 (4,6.4)		4.78 (6,2.4)		
	TT = 4 → 5	TT = 3 → 7		TT = 2 → 6		
		5.74 (4,12.0)				
		TT = 3 → 8				
E						
May and Baker	5.87 (3,2.0)	NP	4.58 (5,0.8)	4.57 (5,1.1)	5.66 (3,15.5)	NP
(L327)	TT = 11 → 15 (MS)		TT = 1 → 3	TT = 1 → 3	TT = 5 → 15 (MS)	
F						
Riedel de Haën	4.95 (5,3.5)	5.13 (4,3.6)				
Hydranal composite 5	TT = 1 → 6	TT = 1 → 8				
Pyridine-free (34805)	4.67 (11,2.8)	4.80 (3,4.9)	4.69 (6,4.4)	4.56 (5,5.2)	4.72 (3,1.4)	4.68 (5,4.6)
	TT = 1 → 8	TT = 1 → 13	TT < 1	TT < 1	TT = 1	TT = 1 → 8
	4.99 (3,3.3)	5.24 (4,17.0)		4.63 (3,4.5)		4.66 (3,3.9)
	TT = 1 → 15 (MS)	TT = 1 → 15 (MS)		TT = 1		TT = 1 → 4

TT = titration time in minutes; NP = not performed; MS = manual stop; Number of titrations and relative standard deviation for each mean value are given in parentheses (N, RSD); catalogue numbers of reagents are mentioned in parentheses.

Table 3
Water content (% m/m H_2O) in an erythromycin sample as obtained with two component Karl Fischer reagents

KF reagent		Independent	Solvent is provided by the manufacturer Consecutive*				
G							
J.T. Baker			TT		TT		
Reaquant		4.50 (5,1.3)	2 → 7	4.45	1		
titrant	(8842)			4.61	1		
solvent "modified"	(8840)			5.16	15 (MS)		
H							
J.T. Baker			TT		TT		TT
Reaquant		4.61 (5,3.0)	1 → 5	4.65	1	4.64	1
titrant	(8842)			4.73	1	4.92	1
solvent "sprint"	(8885)			7.36	15 (MS)	7.02	15 (MS)
I							
BDH			TT		TT		TT
titrant	(19265)	4.53 (5,2.2)	<1	4.32	<1	4.28	<1
solvent	(19266)			4.30	2	4.51	<1
				4.53	1	4.48	<1
				4.50	<1	4.55	<1
				7.40	15 (MS)	6.16	15 (MS)
J							
Riedel de Haën			TT		TT		TT
Hydranal		4.66 (5,1.0)	<1	4.58	<1	4.42	<1
titrant	(34801)			6.20	15 (MS)	6.86	15 (MS)
solvent	(34800)						
K							
Merck			TT		TT		TT
titrant	(9243)	4.51 (5,1.0)	<1	4.53	<1	4.42	<1
solvent	(9241)			4.56	1	5.38	15 (MS)
				6.15	15 (MS)		
L							
Merck			TT		TT		TT
titrant "U"	(9233)	4.53 (5,1.7)	<1	4.56	<1	4.41	<1
solvent	(9241)			4.60	4	4.54	<1
				4.55	<1	4.50	1
				4.67	<1	5.21	10
				4.72	<1		
				4.56	<1		
				6.39	15 (MS)		
M							
Merck			TT		TT		TT
titrant "U"	(9233)	4.63 (5,1.4)	2 →5	4.60	4	4.57	2
solvent "K"	(9221)			4.54	1	4.62	5
				5.40	11	5.09	9

*Each block represents a series of consecutive titrations, each figure represents the result obtained for one titration.

TT = titration in minutes.

MS = manual stop.

Number of titrations and relative standard deviation for each mean value are given in parentheses (N, RSD).

Catalogue numbers of reagents are mentioned in parentheses.

Table 4
Water content (% m/m) in esters of erythromycin and in erythromycin stearate as determined in different solvents with the Ph. Eur. "iodosulphurous reagent"

Solvent	Erythromycin propionate		Erythromycin estolate		Erythromycin ethylsuccinate		Erythromycin stearate	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Methanol ($N = 3$)*	3.24	16.3	4.00	19.8	2.00	25.6	2.78	2.4
Pyridine ($N = 5$)†	2.29	2.2	2.96	0.7	1.63	2.5	2.86	4.1
Pyridine/methanol 1:1 ($N = 5$)†	2.31	2.5	2.94	4.4	1.71	4.2	2.69	2.3

*Independent titrations.
†Consecutive titrations.

As TLC showed the formation of EAEN during the titration with methanol as the solvent, it is believed that decomposition of erythromycin is favoured in solvents which are not or are only slightly buffered. Therefore the KF results and RSD values reported in Table 2 for methanol as the solvent are always higher than those obtained with pyridine. Since the solvent of the USP is not buffered, it gives results comparable to those obtained with methanol. For consecutive titrations in poorly buffered solvents the water content found in each titration is nearly always higher than that found in the former titration. This is due to the continuously increasing amount of erythromycin present in the titration vessel and the formation of EAEN and water by continuous decomposition. This also results in an increase in titration time. A sudden increase in water content and titration time, as observed in Table 3 for consecutive titration, can thus be explained by neutralisation of the solvent buffer and consequent formation of EAEN.

In the same way methanol is not a good solvent for KF titrations of the esters and salts of erythromycin. This is clearly demonstrated in Table 4 with methanol giving higher mean values and variabilities in comparison with pyridine. Values generated with a mixture of pyridine and methanol however are in close agreement with those obtained with pure pyridine. The mean of three independent determinations of water in erythromycin stearate, obtained with methanol as the solvent, is very close to the means found with pyridine or pyridine–methanol (1:1). However, the mean and the RSD of three consecutive determinations were distinctly higher (mean 3.30%; RSD 15.4).

It is believed that in the presence of pyridine the buffer capacity is sufficient to avoid formation of EAEN, in the absence of pyridine or of another suitable buffer the medium is sufficiently acidic to aid the formation of EAEN. From these results it may thus be concluded that the use of pyridine as the solvent allows easy and correct KF titration of water in erythromycin.

Pyridine however has some disadvantages. The time needed to titrate the water present in pure pyridine (blank titration) varies from 2 min to over 15 min for the different KF reagents. The time required to titrate methanol on the other hand usually does not exceed 2 min. Moreover, overtitration during blank titration was sometimes observed with at least two different KF reagents. This overtitration can be avoided by regular cleaning of the platinum electrodes in concentrated nitric acid for about 1 h. Overtitration was probably due to poisoning and subsequent slower response of the electrodes. Titration times for the blank were also somewhat reduced after this cleaning procedure. Another disadvantage is the poor solubility in pyridine of sodium tartrate dihydrate. Therefore standardisation is better performed by titration of a dilution of water in methanol. With methanol as the solvent both these standardisations gave identical results, provided adequate correction was made for the water content of the methanol used for the preparation of the water dilution. The use of pyridine–methanol (1:1) copes with the hitherto mentioned disadvantages since times for blank titrations of the mixture are comparable to those for pure methanol and sodium tartrate dihydrate is sufficiently soluble. A third disadvantage of pyridine is the unpleasant odour. The use of pyridine can be avoided since a 10% m/v solution of imidazole in methanol shows the advantages of a well-buffered solvent (Table 5). Blank titration times are less than 1 min and standardisation with sodium tartrate dihydrate is possible. Titration times for erythromycin are comparable to the titration times obtained with the best commercial reagents. The high titration speed obtained with 10% m/v imidazole in methanol is consistent with the observations made by Verhoef and Barendrecht who reported that the speed of reaction during KF titration is optimal at pH 5.5–8 [7].

Table 5
Mean results (% m/m H_2O, $N = 5$) for consecutive titrations of erythromycin, erythromycin stearate and erythromycin esters as determined with different KF reagents and 10% m/v imidazole in methanol as the solvent

Reagent	Erythromycin Mean (%)	RSD (%)	Erythromycin stearate Mean (%)	RSD (%)	Erythromycin propionate Mean (%)	RSD (%)	Erythromycin estolate Mean (%)	RSD (%)	Erythromycin ethylsuccinate Mean (%)	RSD (%)
A	4.46 TT <1	2.9	2.66 TT <1	3.3	2.20 TT <1	1.6	2.91 TT <1	1.2	1.65 TT <1	4.7
B	4.64 TT = 1 → 2	1.5	NP		NP		NP		NP	
C	4.62 TT <1	2.3	NP		NP		NP		NP	
D	4.62 TT = 1 → 2	1.6	NP		NP		NP		NP	
E	4.52 TT <1	3.5	NP		NP		NP		NP	

TT = titration time in minutes.
NP = not performed.
See Table 2 for reagents.

Table 6
Apparent pH values (pH*) during titration with the Ph, Eur, iodosulphurous reagent in different solvents

	Solvent			
pH*	Methanol	Pyridine	Pyridine:methanol (1:1)	Imidazole (10% m/v) in methanol
before titration: (solvent)	6.5	8.5	8.5	9.3
after titration of the solvent: (blank titration)	4.9	6.2	6.1	7.3
after titration of water (mg) added:				
15 mg	4.9	6.0	5.8	6.7
30 mg	4.7	5.8	5.6	6.2
45 mg	4.6	5.7	5.5	5.7
60 mg	4.6	5.7	5.4	5.4
75 mg	4.6	5.6	5.3	5.2
90 mg	4.6	5.4	5.3	5.2

In order to follow the development of acidity of the medium the pH* was measured before and during titration of dilutions of water in methanol. With methanol as the solvent the pH* drops to lower values than with the other solvents (Table 6).

It is believed that for one component reagents the addition of 10% imidazole to the methanol, used as the solvent, can also improve the KF titration of other pharmaceuticals. The improved buffer capacity enhances the speed of the titration and offers better accuracy. This is especially important when consecutive titrations of an acidic compound are performed.

Conclusion

An accurate KF determination of water in erythromycin and esters or salts of erythromycin is possible. Independent titrations with commercial two component reagents cause no problems but one has to be careful when consecutive titrations are performed. With a one component reagent a buffered solvent should be used. A 10% m/v solution of imidazole in methanol is preferred to pyridine or a mixture of pyridine and methanol. It has good buffer capacity, lacks the unpleasant odour of pyridine and allows a high titration speed.

Acknowledgements: The authors thank Mrs L. Van den Bempt for secretarial assistance.

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[*Received for review 20 October 1987*]