ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1453-1458

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Conductimetric titrimetry for assay of selected antibiotics in non-aqueous media

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Received for review 22 March 1995

Abstract

In this study, a conductimetric titration method is proposed for the determination of some commonly used antibiotics. The conductimetric titration of three antibiotics, namely ampicillin, amoxycillin trihydrate and rifampin, was carried out in acetic acid using perchloric acid as titrant. Ciproflaxacin hydrochloride, however, was titrated after being dissolved in acetic acid containing an excess of mercury(II) acetate. For the titration of netilmicin sulphate, barium acetate prepared in acetic acid was used as titrant. The method was found to be highly accurate and precise, having a relative standard deviation of less than 1.0% for all antibiotics studied. It was also shown that the conductimetric titrimetry could be successfully applied to the assay of commercial preparations containing the above-mentioned antibiotics. The validity of the method was tested by the recovery studies of standard addition to pharmaceuticals and the results were found to be satisfactory.

Keywords: Ampicillin; Amoxycillin trihydrate; Rifampin; Netilmicin sulphate; Ciproflaxacin hydrochloride; Conductimetric titration; Acetic acid solvent; Perchloric acid; Barium acetate; Titrimetric assay

1. Introduction

Ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride are among the most widely used antibiotics. The extensive development of the pharmaceutical field requires more rigorous analytical methods for the control of drugs. Various available methods in the literature for the determinations of these antibiotics include iodometric, mercurimetric titrimetry and spectrophotometry for ampicillin and amoxycillin trihydrate [1-7]; spectrophotometry, chromatography and amperometry for rifampin [8-11]; GLC, TLC and HPLC for netilmicin sulphate [12-14]; chromatography and spectrophotometry for ciproflaxacin hydrochloride [15-18]. When it comes to the routine determinations in pharmaceuticals, the United State Pharmacopeia (USP) prescribes iodometry and chromatography for ampicillin and amoxycillin trihydrate [19], and microbiological methods for rifampin and netilmicin sulphate [20]. However, the USP does not recommend any method for the assay of ciproflaxacin hydrochloride, a relatively new antibiotic.

An inspection of both the available methods and the USP ones for the five above-mentioned antibiotics reveals that most of them are either cumbersome or time consuming, or involve the use of expensive equipment and reagents. However, no reports have appeared dealing with the conductimetric method for the determination of these antibiotics in non-aqueous media so far. Therefore, the aim of the present work is

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SSDI 0731-7085(95)01577-9

to develop a new reliable method as a continuation of our studies related to conductimetric titration in non-aqueous media [21-24]. The proposed conductimetric titration method in acetic acid solvent yields accurate and reproducible results, and has also been applied to the assay of pharmaceutical preparations.

2. Experimental

2.1. Apparatus

An Orion Model 101 conductivity meter equipped with an Orion Cat. No. 99.01.01. conductivity cell (with a cell constant of 1.04 cm^{-1}) was used throughout the study. All titrations were carried out manually in a specially designed cell as described previously [25]. The cell was connected to a water-circulating thermostat at 25 ± 1°C during the titrations.

2.2. Materials

Ampicillin, amoxycillin trihydrate and ciprofloxacin hydrochloride, obtained from Fako Drug Company (Turkey), were of chemically pure laboratory working standard, having purities of 99.5%, 99.0%, and 99.7%, respectively.

Rifampin, obtained from Refik Saydam Hygiene Center (Turkey), was of a chemically pure laboratory working standard, with a purity of 99.9%.

Netilmicin sulphate, received from Eczacıbaşı Drug Company (Turkey), was of a chemically pure laboratory working standard, having a purity of 98.9%.

All the antibiotics were dried in a vacuum oven before being used, except for amoxycillin trihydrate.

Alfasilin (Fako Co.) was labelled as containing ampicillin trihydrate equivalent to 500 mg ampicillin per capsule.

Alfoxil (Fako Co.) was labelled as containing amoxycillin trihydrate equivalent to 500 mg amoxycillin per tablet.

Rifadin (Sifar Co.) was labelled as containing 150 mg rifampin per capsule.

Netromycin (Eczacıbaşı Co.) was labelled as containing netilmicin sulphate equivalent to 150 mg netilmicin base per 1 ml of an ampoule.

Proxacin (Fako Co.) was labelled as containing ciprofloxacin hydrochloride equivalent to 250 mg ciprofloxacin per tablet.

Perchloric acid solution in acetic acid. The anhydrous 0.040 M perchloric acid used in the titration was prepared as follows. 0.80 ml of 70% perchloric acid (Merck) was added dropwise to 5 ml of ice-cooled pure acetic anhydride. The light yellow solution was left for 5-6 h at room temperature. 2.00 ml was then introduced into a 100.0 ml calibrated flask and diluted to volume with acetic acid. This solution was standardized against the primary standard sodium carbonate.

Barium acetate solution in acetic acid. 0.1 M barium acetate solution prepared in glacial acetic acid was gravimetrically standardized by precipitation of the barium ions as barium sulphate.

Glacial acetic acid (pure) was purchased from Merck, and was used after shaking with pure chromium(VI) oxide and subjecting to subsequent fractional distillation [26].

Antibiotics solutions. Ampicillin (6-8 mg), amoxycillin trihydrate (7-8 mg), rifampin (11-14 mg) and netilmicin sulphate (16-21 mg) were dissolved in 25 ml of glacial acetic acid, depending upon their molecular weights. Ciprofloxacin hydrochloride (20-25 mg) was dissolved in 1 ml of 0.1 M mercury(II) acetate solution in acetic acid and then diluted to 25 ml with acetic acid. Aliquots of 20 ml taken from the test solutions of ampicillin, amoxycillin trihydrate, rifampin and ciprofloxacin hydrochloride were titrated with standard perchloric acid solution, at 25°C, under nitrogen atmosphere. The titration of netilmicin sulphate was carried out using standard barium acetate solution as titrant under the same conditions. All the assay solutions were prepared prior to titrations.

2.3. The solutions of pharmaceutical preparations.

Capsules

15 alfasilin capsules were weighed and their average contents were calculated. The contents were pooled and finely powdered, and the required amount of powder was weighed accurately and dissolved in 25 ml glacial acetic acid. The same procedure was applied to rifadin capsules.

Tablets

For both alfoxil and proxacin, 15 tablets were weighed and the average weight was calculated for each. The required amount of finely

grounded alfoxil powder was dissolved in 25 ml of acetic acid, while the required amount of proxacin powder was dissolved in 1 ml 0.1 M mercury(II) acetate solution before being diluted to 25 ml with acetic acid.

Ampoule

An ampoule of netromycin was transferred into a 100 ml standard flask and dissolved in acetic acid to give a stock solution. The required volume of this stock solution was diluted with acetic acid to 25 ml.

The 20 ml aliquots taken from the solutions of the pharmaceutical preparations were also titrated with the above-mentioned titrants under the same conditions as standard antibiotics.

The titrations were repeated for different amounts of each antibiotic and pharmaceutical preparation.

3. Results and discussion

3.1. Determination of standard active components

Ampicillin, amoxycillin trihydrate and rifampin were titrated conductimetrically with perchloric acid as titrant in glacial acetic acid. The titration curves for each of the antibiotics displayed one stoichiometrically well defined end-point. The titration curves of these three compounds are shown in Figs. 1–3. The end-points corresponding to one equivalent of acid in the titration curves of ampicillin and amoxicillin trihydrate can be attributed to the protonation of nitrogen located in the β-lactam ring. However, the end-point corresponding to one equivalent of acid in the titration curve of rifampin can be related to the protonation of $N-CH_3$ in the piperazine ring.

Since ciproflaxacin hydrochloride is not soluble in acetic acid alone, this antibiotic was dissolved using acetic acid containing an excess of mercury(II) acetate [27]. The following reaction was proposed to take place between ciproflaxacin hydrochloride and mercury(II) acetate:

$$2\ \text{Ciproflaxacin.HCl} + \text{Hg}(\text{CH}_3\text{COO})_2 \rightarrow 2\text{Ciproflaxacin} + \text{HgCl}_2 + 2\ \text{CH}_3\text{COOH}$$

Mercury(II) chloride and mercury(II) acetate do not dissociate in acetic acid and therefore did not interfere with the conductimetric titration of this antibiotic; mercury(II) chloride is also readily soluble in acetic acid. The ciproflaxacin formed in the solution was then titrated with perchloric acid in acetic acid. This substance also gave a fairly well shaped stoi-

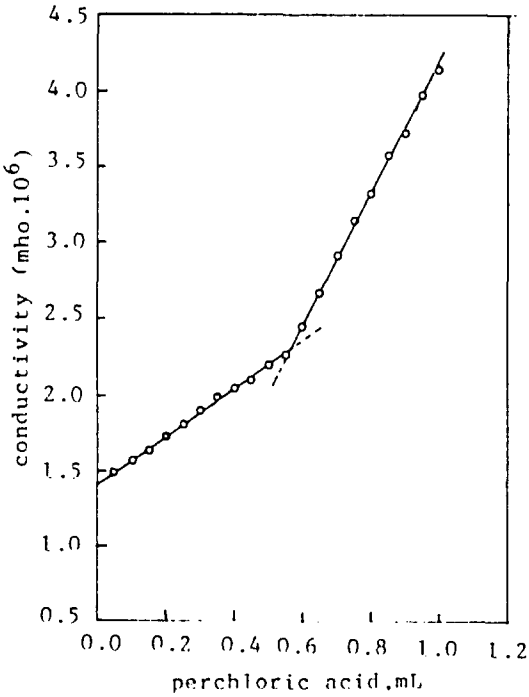

Fig. 1. Conductometric titration curve for ampicillin titrated with perchloric acid in acetic acid solution.

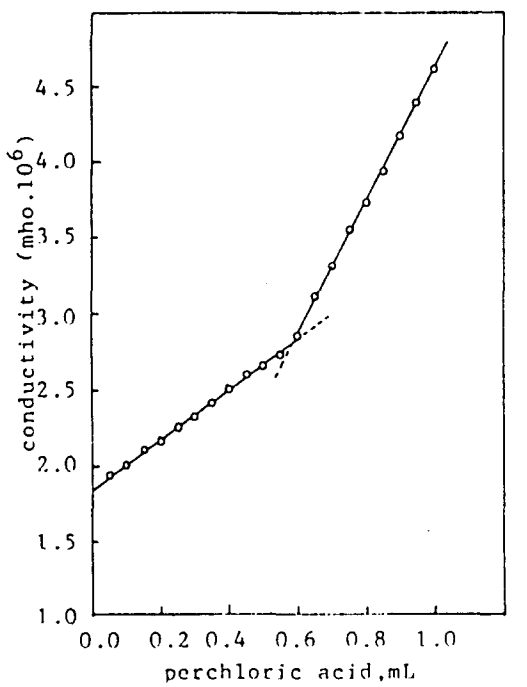

Fig. 2. Conductometric titration curve for amoxcillin trihydrate titrated with perchloric acid in acetic acid solution.

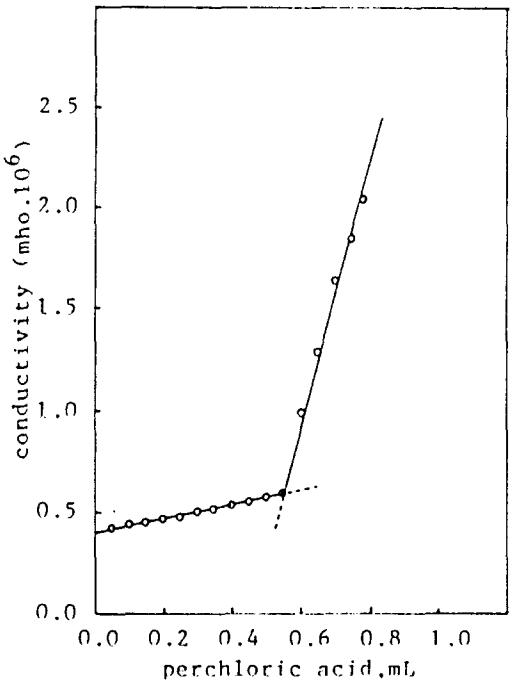

Fig. 3. Conductometric titration curve for rifampin titrated with perchloric acid in acetic acid solution.

chiometric titration curve and showed one stoichiometric end-point corresponding to one equivalent of acid (Fig. 4). The end-point is probably related to the protonation of nitrogen (N H) in the piperazine ring.

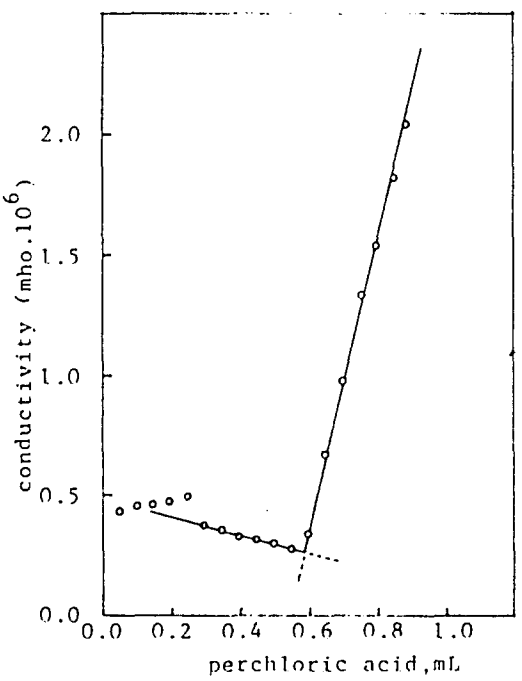

Fig. 4. Conductometric titration curve for ciproflaxacin hydrochloride titrated with perchloric acid in acetic acid solution.

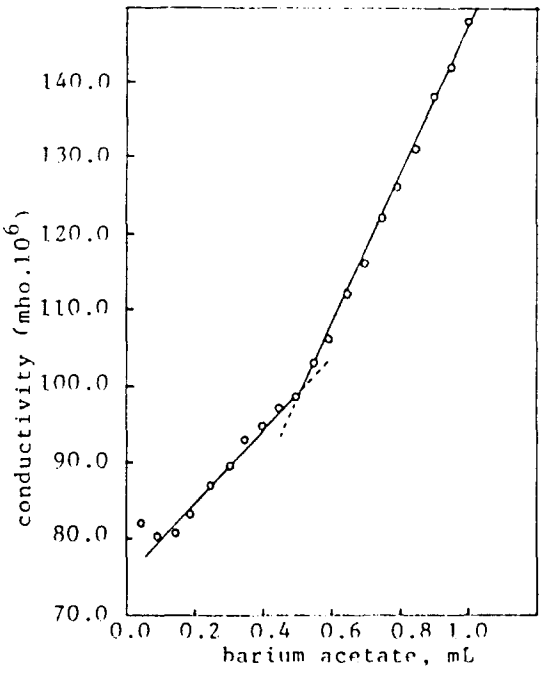

Fig. 5. Conductometric titration curve for netilmicin sulphate titrated with barium acetate in acetic acid solution.

The netilmicin sulphate was titrated using a standard barium acetate solution as titrant in acetic acid. The compound gave only one stoichiometric end-point corresponding to the sum of the sulphates. Thus, 1 mole of netilmicine sulphate was equivalent to 5 moles of barium acetate. The titration curve of netilmicine sulphate is shown in Fig. 5.

The percentage of each antibiotic (chemically pure laboratory working standard) was calculated from the conductimetric titration data. The accuracy and precision of the proposed method were tested by five successive determinations performed on ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride. The results are given in Table 1.

When the data in Table 1 are examined, the mean values obtained by the conductimetric method are in good agreement with the nominal values given for each antibiotic, and the relative standard deviations (RSDs) are less than $\pm 1\%$. This indicates that the accuracy and precision of the proposed method are quite satisfactory. Further, the comparison of the results in this study with those obtained for the same antibiotics by a potentiometric method in our previous work [28] indicates that the proposed conductimetric method could be utilized as safely as the potentiometric method in the determination of ampicillin, amoxycillin trihy-

Table 1
Titrimetric determination on antibiotics which are chemically pure laboratory working standards

Antibiotics	Proposed method[a]		Nominal value (%)
	Mean (%)	RSD	
Ampicillin	98.7	0.91	99.5
Amoxycillin trihydrate	98.9	0.97	99.0
Rifampin	99.6	0.72	99.9
Netilmicin sulphate	98.7	0.25	98.9
Ciproflaxacin hydrochloride	99.9	0.46	99.7

[a]Mean and relative standard deviation for five determinations.

Table 2
Titrimetric determination of antibodies in some pharmaceutical preparations

Pharmaceuticals	Antibiotics	Recovery (% ± RSD)[a]
Alfasilin	Ampicillin	102.30 ± 0.73
Alfoxil	Amoxycillin trihydrate	100.52 ± 0.80
Rifadin	Rifampin	101.73 ± 0.82
Netromycin	Netilmicin sulphate	100.35 ± 0.97
Proxacin	Ciproflaxacin hydrochloride	99.97 ± 0.28

[a]Mean and relative standard deviation for five determinations. Recovery relative to nominal content.

drate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride.

3.2. Determination of the active components in pharmaceutials

In order to evaluate the applicability of the conductimetric method to pharmaceutials, the determinations of ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride were carried out in alfasilin, alfoxil, rifadin, netromycin and proxacin, respectively, under the same conditions as employed for the pure antibiotics. The fact that the shapes of the conductimetric titration curves of pure antibiotics and their corresponding pharmaceuticals are nearly the same proves that the excipients which might be present in

Table 3
Recovery studies of standard additions to some pharmaceutical preparations

Pharmaceuticals	Antibiotics	Added (mg)	Found (mg)	Recovery (%)[a]
Alfasilin	Ampicillin	3.00	2.98	99.33
		4.00	3.94	98.50
		5.00	5.01	100.20
Alfoxil	Amoxycillin trihydrate	3.00	3.03	101.00
		4.00	4.00	100.00
		5.00	5.06	101.20
Rifadin	Rifampin	6.00	6.02	100.33
		8.00	7.93	99.13
		10.00	10.04	100.40
Netromycin	Netilmicin sulphate	2.00	2.00	100.00
		2.50	2.51	100.40
		3.00	3.04	101.33
Proxacin	Ciproflaxacin hydrochloride	2.00	1.99	99.50
		2.50	2.47	98.88
		3.00	3.00	100.00

[a]Ampicillin: mean ± RSD = 99.34 ± 0.85. Amoxycillin trihydrate: mean ± RSD = 100.73 ± 0.64. Ripampin: mean ± RSD = 99.95 ± 0.71. Netilmicin sulphate: mean ± RSD = 100.58 ± 0.68. Ciproflaxacin hydrochloride: mean ± RSD = 99.46 ± 0.56.

the pharmaceutical preparations do not effect the titration curves. However, no work has been done to test the effects of degradation products. Thus, the method cannot be considered to be stability-indicating for these antibiotics.

Table 2 summarizes the results obtained for each antibiotic in the corresponding pharmaceuticals, expressed as percentages of the nominal contents. The recoveries agree well enough with the nominal contents and the RSD values are less than $\pm 1\%$. Thus, the precision of the method is very satisfactory for the determination of ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride in corresponding pharmaceutical dosage forms. The results clearly demonstrate the utility of the proposed conductimetric titrimetry for analysis of the each antibiotic in pharmaceuticals.

The recovery studies of standard additions to commercial pharmaceuticals were also carried out to provide further support for the validity of the method. The related data are given in Table 3. The mean percentage recoveries and their relative standard deviations were found to be 99.34–100.73% and 0.56–0.85% respectively. These results also confirm the precision and the validity of the conductimetric method.

As a result of this study, the proposed method can be successfully applied to the determination of ampicillin, amoxycillin trihydrate, rifampin, netilmicin sulphate and ciproflaxacin hydrochloride, and the analysis of some of their pharmaceutical preparations. In conclusion, the simplicity, accuracy and precision of the proposed conductimetric titrimetry indicate that this method is quite suitable for routine quality control analysis of pharmaceutical preparations. In addition, it is highly cost and time effective.

Acknowledgement

The authors would like to thank the Faco Drug Co., The Eczacıbaşı Drug Co. and Refik Saydam Hygine Centre for providing pure antibiotics.

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