Journal of Pharmaceutical & Biomedical Analysis
Vol. 3, No. 4, pp. 367–370, 1985
Printed in Great Britain

0731–7085/85 $3.00 + 0.00
Pergamon Press Ltd.

Short Communication

Rapid microbiological pH assay for the determination of cephradine in pharmaceutical formulations and biological fluids

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Keywords: *Bioassay by ΔpH;* Staphylococcus aureus *ATCC 6538P; cephradine in biological fluids; cephradine in pharmaceutical formulations.*

Introduction

Methods of analysis for cephradine include microbiological [1], fluorimetric [2], UV spectroscopic, HPLC, iodometric and hydroxylamine procedures [3]. These existing methods either have complex sample preparations and use sophisticated equipment or require long incubation times. An alternative simple and rapid method would have advantages, particularly in the clinical situation.

Classical microbiological assays suffer from the variability of results and the relatively long incubation periods required for the organism to elicit its response. Rapid and automated methods, many based on the measurement of physicochemical parameters rather than the measurement of growth or no growth, have much to contribute to the rapidity and improvement of microbiological assays. A constant source of inocula from liquid nitrogen storage [4] is of importance in these assays, providing microbiological cells that have known responses to specified antibiotics and can be used over a number of years without decrease of viability. Faine and Knight [5] reported an assay for kanamycin and gentamicin based on the pH change caused by a rapidly metabolizing organism. Since the appearance of this method, the ready availability of reliable inocula and much improved pH meters and electrodes offer considerable scope for the development of this type of assay.

The products of metabolism of a microorganism produce a pH change in the growth medium. An antibiotic will either reduce or prevent the processes of growth, and the interaction between sensitive organism and antibiotic is proportional over a certain concentration range. *Staphylococcus aureus* ATCC 6538P, when grown in an unbuffered

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medium, produces large amounts of acids which cause reproducible pH changes over 5 h incubation at 37°C. This organism is particularly suitable for these studies as it has a short generation time and possesses minimal β-lactamase activity. The change in pH is proportional to the concentration of cephalosporin present in the growth medium and is the basis of the method reported here.

Experimental

Apparatus and media

Tryptone Soya Broth (TSB) without KH_2PO_4, was used throughout with the following formula per litre: tryptone, 17.0g; neutralized soya peptone, 3.0g; D-glucose, 2.5g and sodium chloride, 5.0g.

The pH was adjusted to pH 7.60 with a few drops of 10 M sodium hydroxide. Tryptone and neutralized soya peptone were obtained from Oxoid Ltd, Basingstoke, Hants, and D-glucose and sodium chloride were obtained from British Drug Houses Ltd, Poole, UK. Media were dispensed using an automatic diluter (Hamilton, Bonaduz, Switzerland) into standard 1 oz bottles. After incubation, pH measurements were made using a Philips digital ion activity meter PW 9414 fitted with a standard hydrogen ion electrode.

Materials

Serum and urine were obtained from pooled human sources, dispensed in 20 ml volumes and stored at −20°C. Cephradine and cephradine formulations (Velosef) were house standards and market packs (E.R. Squibb, Moreton, Wirral, UK).

Inoculum preparation

A liquid nitrogen stored inoculum was prepared as previously described [4]. The optimum cell concentration for the assay was 10^9 CFU ml^{-1}. Alternatively, fresh overnight cultures of the organism were grown in TSB at 37°C. After three saline washes (0.9% w/v), cells were diluted in saline (0.9% w/v) to approximately 10^9 CFU ml^{-1}.

Procedure

(i) *Biological samples.* Standards were diluted in serum or urine to give concentrations of 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 μg ml^{-1} from a freshly prepared cephradine stock solution of 250 μg ml^{-1} in the appropriate biological fluid. Samples were diluted in control serum or urine to a concentration approximating the midpoint of the standard range. Both standards and samples were further diluted 1 in 10 in inoculated assay broth.

(ii) *Pharmaceutical samples.* Standards were diluted in distilled water to give concentrations of 250, 125, 62.5, 31.25, 15.6 and 7.8 μg ml^{-1} from a freshly prepared cephradine stock solution of 250 μg ml^{-1} in distilled water. Samples were diluted in distilled water to a concentration approximating the mid-point of the standard range. Both standards and samples were further diluted 1 in 100 in inoculated assay broth.

(iii) *Assay.* Assay broth was inoculated with *Staphylococcus aureus* ATCC 6538P to give approximately 5×10^6 CFU ml^{-1}. Standards and samples were prepared in triplicate using an automatic diluter, dispensed in 10 ml volumes in 1 oz capped bottles and placed in racks. Incubation was for 5 h at 37 ± 0.5°C in a water bath with vigorous

shaking. After incubation, all assay bottles were rapidly cooled to 4°C by placing in a pre-cooled water bath to reduce further pH change. The pH of the contents of each assay bottle was determined within 10 min by removal of the cap and introduction of a pre-calibrated pH electrode. A typical pH profile after 5 h incubation at 37°C for the assay of cephradine in finished products is shown in Fig. 1.

Discussion

The accuracy, precision and sensitivity of the ΔpH method are demonstrated by the close correlation of results in Table 1. These parameters are compared with those from agar diffusion when assaying cephradine-containing products in Table 2. They demonstrate no significant difference in accuracy between the two methods, but improved precision for the ΔpH method. Additionally, the reproducibility of this technique is improved by the use of liquid nitrogen stored inocula instead of freshly prepared inocula with their inherently variable characteristics. Coupled with the increased speed and sensitivity of the ΔpH method over traditional plate diffusion techniques, and since turbid samples do not affect the result as they do in the turbidimetric type of assay, the method provides a rapid and sensitive assay for cephradine in both dosage forms and biological samples. In addition, with no need for sophisticated and expensive equipment, the assay lends itself to use in small laboratory situations, and the simplicity of the steps involved could well be fully automated with minimal effort.

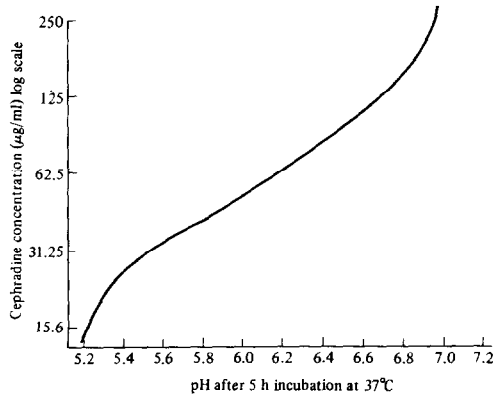

Figure 1
Typical $\Delta pH_{(5h)}$ profile for the assay of cephradine in finished products.

Table 1
Accuracy and precision of analysis for spiked cephradine samples in serum or urine

Sample	Theoretical cephradine concentration ($\mu g\ ml^{-1}$)	Mean result ± SD (n=6) ($\mu g\ ml^{-1}$)	Theoretical concentration (%)	RSD (%)
Serum	20.00	20.57 ± 1.02	102.9	5.0
Serum	5.00	4.89 ± 0.38	97.8	7.8
Serum	0.50	0.48 ± 0.02	96.0	4.2
Urine	100.00	102.00 ± 2.97	102.0	2.9
Urine	20.00	20.50 ± 0.63	102.5	3.1
Urine	5.00	5.13 ± 0.11	102.6	2.1

Table 2
Accuracy and precision of analysis of cephradine containing products by both ΔpH and agar diffusion methods

Sample	Theoretical cephradine concentration (mg/dose)		Mean result ± SD (n=6) (mg/dose)	Theoretical concentration (%)	RSD (%)
Cephradine capsules	250 mg/cap	ΔpH	255.8 ± 7.69	102.3	3.01
		Diffusion	260.3 ± 11.22	104.1	4.31
Cephradine capsules	500 mg/cap	ΔpH	506.7 ± 17.70	101.3	3.49
		Diffusion	511.2 ± 13.43	102.2	2.63
Velosef for injection	500 mg/inj.	ΔpH	526.1 ± 6.22	105.2	1.18
		Diffusion	529.9 ± 23.96	106.0	4.52
Velosef for injection (argine blend)	500 mg/inj.	ΔpH	500.5 ± 7.09	100.1	1.42
		Diffusion	513.8 ± 25.18	102.8	4.90
Velosef syrup	250 mg/5 ml	ΔpH	256.4 ± 2.77	102.6	1.08
		Diffusion	265.4 ± 10.32	106.2	3.89

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[*Received 21 April 1985*]