ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 1273–1278

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

UV-spectrophotometric determination of ampicillin sodium and sulbactam sodium in two-component mixtures

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Accepted 17 November 1997

Abstract

A simple spectrophotometric method is used for the resolution of the binary mixtures of ampicillin sodium and sulbactam sodium. In aqueous solution, zero-order spectra are subject to interference, so first-derivative spectrophotometry was used to enhance the spectral details allowing the determination of ampicillin sodium from the signal at the zero-crossing point for sulbactam sodium at 268 nm. In 0.1 N sodium hydroxide, sulbactam sodium was determined from the absorbance at 260 nm with negligible contribution from ampicillin sodium. Also, sulbactam sodium was determined without interference using first- and second-derivative spectra in 0.1 N sodium hydroxide at 276 nm (peak-height) and 262–284 nm (peak-to-peak), respectively. The method is rapid, simple, does not require a separation step and allows the determination of each drug without interference from the other. The proposed method has been applied successfully to the assay of these drugs in mixtures and in commercial injections.

Keywords: Ampicillin sodium; Sulbactam sodium; Pharmaceutical combinations; Derivative spectrophotometry

1. Introduction

Sulbactam sodium is available in combination with ampicillin sodium for injection in a 1:2 ratio (w/w). Sulbactam sodium is a competitive, irreversible beta-lactamase inhibitor. Synergy with beta-lactam antibiotics is most marked in bacterial species in which beta-lactamase is a major mechanism of resistance [1].

In spite of the increasing use of this mixture in the treatment of urinary tract and respiratory tract infections, little has been reported concerning its analysis in pharmaceutical preparations.

Sulbactam was determined in serum in presence of ampicillin by synergic bioassay, GC–MS and HPLC methods [2]. The United States Pharmacopoeia [3] reported a simultaneous high-performance liquid chromatographic method for the analysis of sterile ampicillin sodium and sulbactam sodium in injection. A good spectrophotometric method for the simultaneous assay would be of some interest.

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0731-7085/98/$ - see front matter
PII S0731-7085(97)00273-2

Table 1
Assay parameters for the spectrophotometric determination of ampicillin sodium and sulbactam sodium combination

Drug	Solvent	Method	Wavelength (nm)	Conc. range (μg ml^{-} 1)	Regression equation (r)	RSD[a] (%)
Ampicillin sodium	Dist. water	D_1	268	20–100	$D_1 = -0.008 + 0.614C$ (0.9999)	0.44
Sulbactam sodium	0.1 NaOH	A	260	2–16	$A = 0.011 + 0.068C$ (0.9999)	1.26
		D_1	276	2–16	$D_1 = 1.750 + 7.375C$ (0.9999)	1.76
		D_2	262–284	2–16	$D_2 = 0.510 + 4.970C$ (0.9999)	0.50

[a]Average of six separate determinations.

Derivative spectrophotometry is an analytical technique of great utility for resolving some mixtures of compounds with overlapping spectra [4,5]. The principles and advantages of this technique have been described by O'Haver and Green [6]. The use of derivative spectrophotometry has grown spectacularly over the last few years, especially in pharmaceutical, clinical and biochemical, as well as in inorganic and organic analysis [7].

The present paper describes a derivative spectrophotometric method for the simultaneous determination of ampicillin sodium and sulbactam sodium in synthetic mixtures and in commercial injections without prior separation of the two antibiotics.

2. Experimental

2.1. Apparatus

A Pye Unicam PU 8800 UV/VIS spectrophotometer (Philips) with 1-cm quartz cells was used. Suitable settings were:

1. For ampicillin sodium determination. Mode, D_1 spectra over the range 300–230 nm; scan speed, 1 nm s^{-1}; bandwidth, 2 nm; recorder scale, 10 nm cm^{-1}, response time, 20 s and span, 5.
2. For sulbactam sodium determination: Mode, D_1 and D_2 spectra over range 320–230 nm; scan speed, 1 nm s^{-1}; bandwidth, 2 nm; recorder scale, 20 nm cm^{-1}; response time, 20 s and span, 20 for D_1 mode and 2 for D_2 mode.

2.2. Reagents and materials

Sodium hydroxide solution (0.1 N) (Prolabo, France); ampicillin sodium (Cid, Cairo, Egypt); sulbactam sodium (Pfizer, USA); Unasyn injection (Pfizer, Egypt) was purchased from commercial sources.

2.3. Construction of calibration graphs

Prepare stock solutions of ampicillin sodium and sulbactam sodium (0.5 mg ml^{-1} and 0.25 mg ml^{-1}, respectively) in distilled water. Prepare six serial dilutions for each compound within the concentration range stated in Table 1 using distilled water and 0.1 N sodium hydroxide for ampicillin sodium and sulbactam sodium, respectively.

1. For ampicillin sodium determination. Record the first-derivative spectra against distilled water. Measure the D_1-values (peak-height) at the selected wavelength (Table 1).
2. For sulbactam sodium determination. After 15 min from the addition of 0.1 N sodium hydroxide, measure the absorbance and record the first- and second-derivative spectra against 0.1 N sodium hydroxide. Measure the D_1-values (peak-height) and the D_2-values (peak-to-peak) at the selected wavelengths (Table 1).

2.4. Procedures for Unasyn injection

Transfer an accurately weighed amount of the mixed contents of ten vials equivalent to 100 mg of ampicillin sodium and 50 mg of sulbactam

sodium into a 100-ml volumetric flask. Dilute to the mark with distilled water and sonicate for 5 min. Transfer 1.5 ml and 0.5 ml portions into two separate 25-ml volumetric flasks and dilute with distilled water (for ampicillin sodium determination) and 0.1 N sodium hydroxide (for sulbactam sodium determination), respectively. Proceed as described under calibration graphs. Calculate the concentration of each drug using either the calibration graph or regression equation (Table 1).

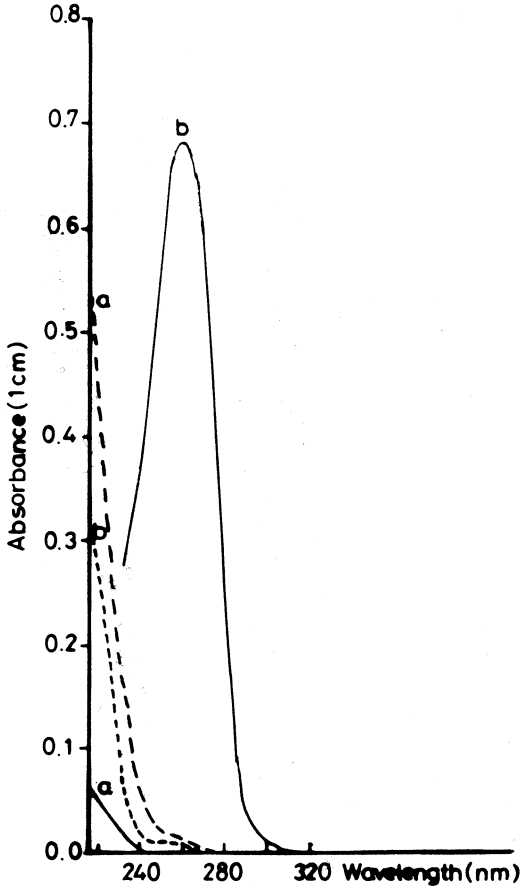

Fig. 1. Zero-order spectra of ampicillin sodium, 20 μg ml^{-1} (- - -) and sulbactam sodium, 10 μg ml^{-1} (——) in water (a) and 0.1 N sodium hydroxide (b).

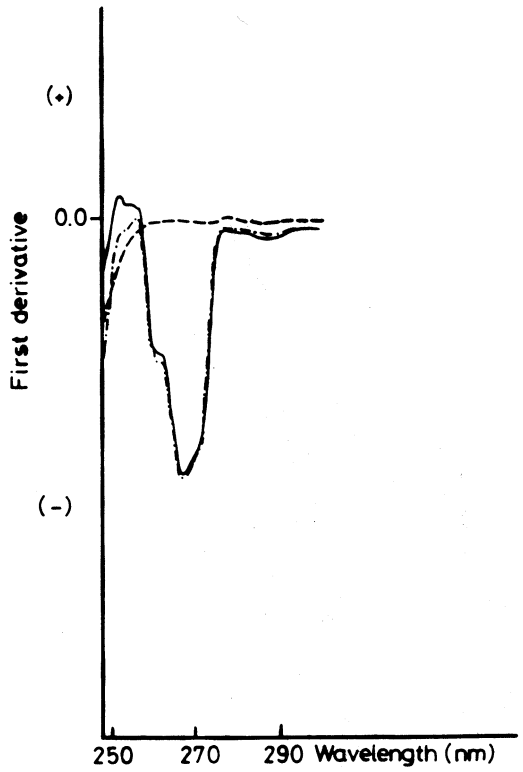

Fig. 2. First-derivative spectra of ampicillin sodium, 100 μg ml^{-1} (——); sulbactam sodium, 50 μg ml^{-1} (- - -) and a mixture of ampicillin sodium, 100 μg ml^{-1} and sulbactam sodium, 50 μg ml^{-1} (– · –) in water.

3. Results and discussion

Ampicillin is 6-[D(-)-∝-aminophenyl-acetamido] penicillanic acid. Sulbactan is penicillanic acid sulfone, (2*S*,5*R*)-3-3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic 4,4-dioxide. The only chromophore in the two molecules is the isolated phenyl group in ampicillin. Making use of this, a good first-derivative method is developed for its selective determination.

Fig. 1 shows the zero-order spectra of ampicillin sodium and sulbactam sodium in water. Fig. 2 shows the first-derivative spectra of both compounds in water. Ampicillin sodium shows a well defined maximum at 268 nm while sulbactam sodium has no contribution above 250 nm. Both compounds show considerable overlap below 250

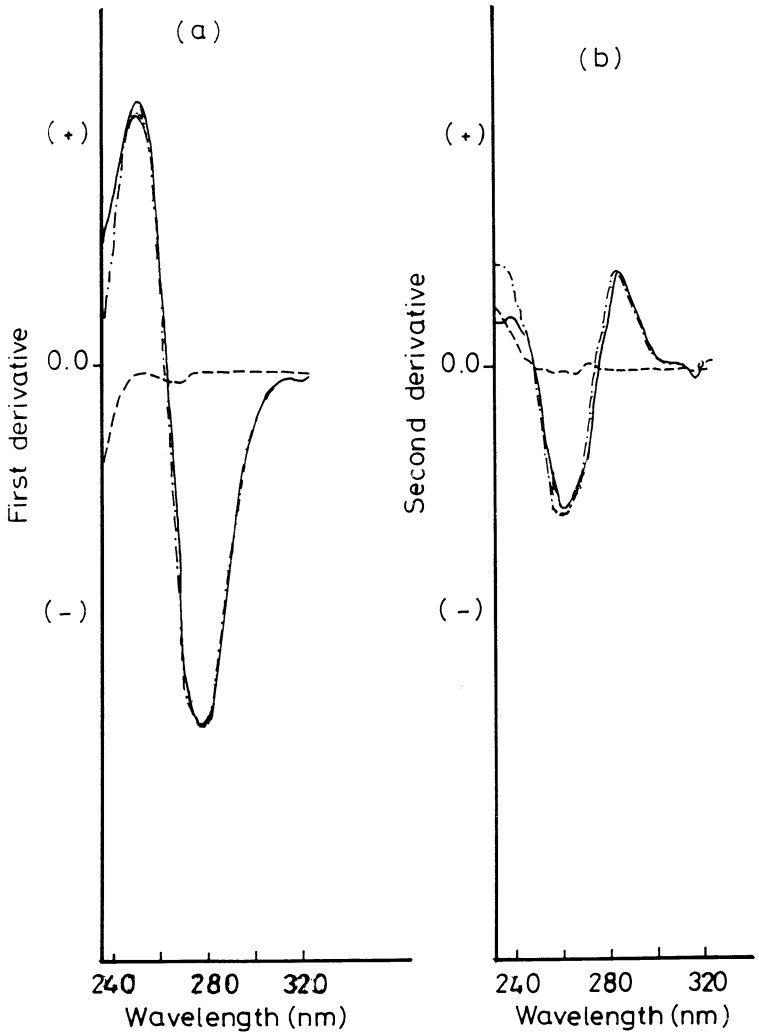

Fig. 3. First-derivative (a) and second-derivative (b) spectra of ampicillin sodium, 20 μg ml^{-1} (- - -), sulbactam sodium, 10 μg ml^{-1} (——) and a mixture of ampicillin sodium 20 μg ml^{-1} and sulbactam sodium, 10 μg ml^{-1} (– · –) in 0.1 N sodium hydroxide.

Table 2
Assay results for the determination of ampicillin sodium and sulbactam sodium in synthetic mixtures

Drug	Amount added (μg ml^{-1})		% Recovery		
	Ampicillin sodium	Sulbactam sodium	A	D_1	D_2
Ampicillin sodium	30	30		100.51	
	60	120		99.95	
	30	90		100.51	
	20	10		100.26	
	100	50		100.21	
	90	30		99.58	
Mean ± S.D.				100.17 ± 0.33	
Sulbactam sodium	20	10	100.21	100.55	99.97
	30	10	100.05	100.41	100.24
	10	10	101.00	100.95	100.80
	5	10	99.70	100.72	100.44
	4	12	100.40	100.53	100.00
	20	16	100.25	100.08	100.57
Mean ± S.D.			100.27 ± 0.39	100.54 ± 0.27	100.34 ± 0.30

Table 3
Assay results for the determination of ampicillin sodium and sulbactam sodium in commercial injection

Sample No.	% Found			
	Ampicillin sodium	Sulbactam sodium		
	D_1	A	D_1	D_2
1	100.52	101.15	101.01	100.78
2	101.37	100.30	100.90	100.56
3	101.30	100.00	99.41	100.04
4	100.63	101.41	100.37	100.62
5	99.87	100.20	101.85	101.39
Mean ± S.D.	100.74 ± 0.55	100.61 ± 0.56	100.71 ± 0.80	100.68 ± 0.43

nm. Thus the D_1-mode allows the determination of ampicillin sodium in presence of sulbactam sodium by measuring the D_1-values of the minimum at 268 nm (peak-height).

The basis for the determination of sulbactam is that in strongly alkaline solution this fully saturated and spectrophotometrically inactive material rapidly develops an absorbance maximum around 260 nm. Kemal and Knowles [8] reported that 5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoic acid, which shows UV absorption around 267 nm, might be formed from sulbactam enzymatically with TEM-2β-lactamase from *Escherichia coli*, and non-enzymatically at high pH. Haginaka et al. [9] presented an HPLC method with post-column alkaline-degradation for the determination of sulbactam in urine and plasma.

In the present work, degradation of sulbactam sodium was effected using 0.1 N sodium hydroxide at room temperature. On studying the effect of time on the chromophormic product, it was found that maximal stability was attained within 15 min and the product remained stable for at least 1 h. Similarly, the spectrum of ampicillin in 0.1 N sodium hydroxide was studied and showed no spectral changes.

Fig. 1 shows the zero-order spectra of ampicillin sodium and sulbactam sodium in 0.1 N sodium hydroxide recorded after 15 min. Their corresponding first- and second-derivative spectra are shown in Fig. 3. Sulbactam sodium shows a strong absorption band at 260 nm [$\epsilon \cong 17000$] with negligible contribution from ampicillin sodium (Fig. 1). The D_1-spectra show two D_1-signals for sulbactam sodium at 250 and 276 nm, while ampicillin sodium possesses a slight and zero contribution at these signals, respectively. The D_2-spectra show a minimum and maximum for sulbactam sodium at 262 and 284 nm, respectively. However, ampicillin sodium has a negligi-

Table 4
Recovery of ampicillin sodium and sulbactam sodium added to injections

Ampicillin sodium		Sulbactam sodium			
Added[a] (μg ml^{-1})	% Recovery	Added[a] (μg ml^{-1})	Recovery		
	D_1		A	D_1	D_2
20	99.34	2	99.76	99.95	100.31
30	99.20	4	100.60	100.27	100.29
40	100.63	6	100.13	100.12	100.24
50	100.51	8	99.46	101.00	100.56

[a]Each added to a dilution from the commercial injection containing 16:8 (μg ml^{-1}) ampicillin sodium/sulbactam sodium.

ble D_2-contribution in the wavelength range 262–284 nm. Therefore, sulbactam sodium can be quantified without interference from ampicillin sodium using the A-value at 260 nm, the D_1-values (peak-height) at 276 nm and the D_2-values (peak-to-peak) at the wavelength range 262–284 nm, respectively.

Under the described experimental conditions, the graphs obtained by plotting A, D_1 and/or D_2 values versus concentration (in the range cited in Table 1) show linear relationships. Using the method of least squares, regression equations and correlation coefficients for the different calibration data were calculated and presented in Table 1. Separate determinations at different concentrations levels were carried out for each drug to test reproducibility of the A, D_1 and/or D_2 values. The relative standard deviations (RSD) were found to be less than 2% (Table 1).

To prove the validity and the applicability of the proposed method six synthetic mixtures of different ratios in the concentration range stated in Table 2 were assayed. The results obtained were precise and accurate over the different proportions of drug components analysed by the proposed methods (Table 2).

The method was further applied to the determination of ampicillin sodium and sulbactam sodium in Unasyn injection, which is a simple binary mixture with no added excipients. The results obtained (Table 3) for both compounds were in good agreement with the label claims. In order to verify the accuracy of the described method, recovery experiments by the standard addition method were carried out. The results obtained (Table 4) showed satisfactory recovery and confirmed the accuracy of the method.

In conclusion, the proposed method is simple as there is no need for solvent extraction and separation as it estimates each drug independent of the other. Furthermore, the successful application of the proposed method confirms that derivative spectrophotometry offers accuracy and precision for the determination of each drug without interference from the co-existing drug.

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