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PMR DETERMINATION OF A MIXTURE OF AMPICILLIN AND DICLOXACILLIN IN DOSAGE FORM

Momdouh M.F. Metwally

Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

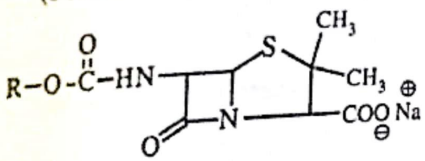
ABSTRACT

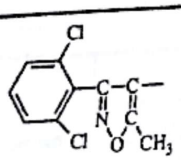
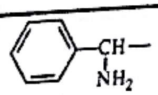
Pmr technique has been used to determine quantitatively ampicillin and dicloxacillin. This was conducted by the use of maleic anhydride as internal standard and by measuring the integrating response of the sharp singlet, the two hydrogen of maleic anhydride that appear at δ=6.55 ppm and compared to the peak height of the two aromatic hydrogen groups of ampicillin and dicloxacillin that integrate for 8H atoms that appear as doublet at 8.0 ppm. The ampicillin content of the mixture is being determined by measuring the integrating response of the peak height of the doublet of the 7H of ampicillin. The mean % recovery ± S.D. were (101.15 ± 1.63), (99.68±2.92) and (99.62±5.86) for ampicillin, dicloxacillin and total respectively.

INTRODUCTION

Penicillins are among the oldest and most frequently prescribed drugs of the naturally occurring antibiotics. They are commonly referred to as the β-Lactam antibiotics.

Table (1): Chemical Structures of Penicillins Examined (6-Amino Penicillanic Acid)



Penicillin	R
Dicloxacillin, Na	
Ampicillin, Na	

Various analytical techniques have been proposed for the determination of ampicillin and dicloxacillin. These include titrimetric method[1] for determination of penicillins containing free primary amino group, U.V. and visible spectrophotometric methods[2-5]. Other are colorimetric methods[6-9], I.R. spectroscopic methods[10-13] fluorometric methods[14-17], and spectrophotometric methods involving the oxidation using NH_4VO_3 to determine all other derivatives[18].

EXPERIMENTAL

Reagents:

- Penicillins: Reference materials.
- Ampicillin-Cid Chemical Co., Egypt, FW=371.39, m.p. = 215°C.
- Dicloxacillin-Cid Chemical Co., Egypt, FW = 493.39
- DMSO-d_6-Sigma Chemical Co. U.S.A. Ampoule Labelled to contain 1.0 ml of solvent.
- Maleic anhydride: Aldrich Chemical CO., FW=90.06, m.p.=45-56°C.
- Apparatus: All pmr spectra were recorded on a Varian EM-390 90-MHz spectrometer.

Methods:

A- for the assay of authentic drugs:

Different specified amounts of ampicillin and dicloxacillin (5.....100mg) of both drugs were accurately weighed into glass stoppered weighing bottles containing each 5.0 mg of maleic anhydride, then sufficient specified amount of deuterated dimethyl suloxide (DMSO-d_6) was added to each bottle which was shaken vigorously with aid of warming in water bath at 50°C±0.5. The solution was filtered, and the clear filtrate was transferred into NMR tube to run the spectra. The Peaks at δ = 1.39 ppm, δ = 1.43 ppm, δ = 4.2 ppm, δ = 2.95 ppm, δ = 8.0 ppm, and at δ = 6.5 ppm, were integrated three times through three experiments, then the average sum of integrals of peaks were determined.

The above mentioned peaks were used to calculate the concentration of total drug mixture and individual drug form. The calculation was carried out as described in previous methods[19].

RESULTS AND DISCUSSION

The ^1H nmr spectrum of maleic anhydride showed a sharp singlet peak at 6.5 ppm. While the ^1H nmr spectrum of ampicillin showed a sharp two Singlet at

1.39 and 1.43 ppm due to the two methyl groups which are quite separated fro mthe other protons that allow the determination of ampicillin, fortunately the ^1H nmr spectrum of dicloxacillin shows the same sharp two signlet at 1.39 and 1.43 the sum integral of the 12 H (4 methyl groups) can be sued for determining the total mixture ampicillin and dicloxacillin.

Ampicillin showed a peak height intergral of the 1H at δ 4.2 ppm (s) due to H residue in the heterocyclic structure of ampicillin which is somewht clear and isolated from other protons that can be used for determination of ampicillin only.

Dicloxacillin showed a peak height integral of the CH_3 methyl group in isoxazole residue appears as singlet integrating for 3H at δ=2.95 ppm is measured which is quite far from the internal standard.The maleic anhydride is freely soluble and inert toward the drugs being estimated.

The pmr spectrum of the mixture of both drugs ampicillin and dicloxacillin with the internal standard maleic anhydride is shown in fig. (4). The results of the experiments were tabulated in tables (3, 4, 5).

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Statistical analysis of the results:

(1) Ampicillin:

The ratio of the weight of drug (ampicillin) and the weight of internal standared (maleic anhydride) to the ratio of the peak height (integral detector response) of the ampicillin and the peak height (intergral detector response) of the internal standard shows a linear relationship with r=0.999 and r^2=0.999.

The regression linear equation:

$Y = 9.02 \times 10^{-5} + 0.121\ x$

where x = ratio of w_1/w_2

Y = ratio of peak height integeral detecter response of D1/S

D1 = drug integeral response in mm

S^1 = standard integeral response in m.m.

The logarithmic regression equation:

$\ln Y = \ln. 4.99 \times 10^{-2}\ e^{0.866x}$

or $Y = 0.13 + 0.22 \ln x.$

The power regression equation:

$Y = 0.12\ x^{0.99}$ or & $\ln. Y = \ln 0.12 + 0.99 \ln X$

(2) Dicloxacillin sodium:

The linear regression equation is:

$Y = 5.4 \times 10^{-2} = 0.27\ X.$

The exponential regression equation is:

$\ln Y = \ln. 0.24 + 0.39\ X$

Table (2) : PMR of the protons of ampicillin, dicloxacillin and maleic anhydride in DMSO-d_6 solvent.

Maleic anhydride		Ampicillin		Dicloxacillin	
Chemical shift ppm	Multiplicity and assignment	Chemical shift ppm	Multiplicity & assignment	Chemical shift ppm	Multiplicity & assignment
6.5 (s)	2H (s)	1.43 (s)	3H α-CH_3 (3H)	1.43 (s)	3 α CH_3 (3H)
		1.39 (s)	3H β-CH_3 (3H)	1.39 (s)	3 β CH_3 (3H)
		5.39 q	1H (H5)	4.81 (s)	1H (H3)
		9.0	1H (H6)	5.46 (d)	1H (H5)
		5.95 b	1H (H7)	5.62 (d)	1H (H6)
		4.84 (s, br)	1H (H8)	7.5 (dd)	3H (aromatic)
		7.36	5H (H9) aromatic	2.95 (s)	3H (isoxazole 5)
		4.64 b	2H (H 10) NH_2	4.0 (s)	1H (H2)
		2.5 (s)	1H H2)		
		4.0 (s)			

s = singlet. b= broad. m= multiplet q= quartet. d= doublet. dd= double doublet

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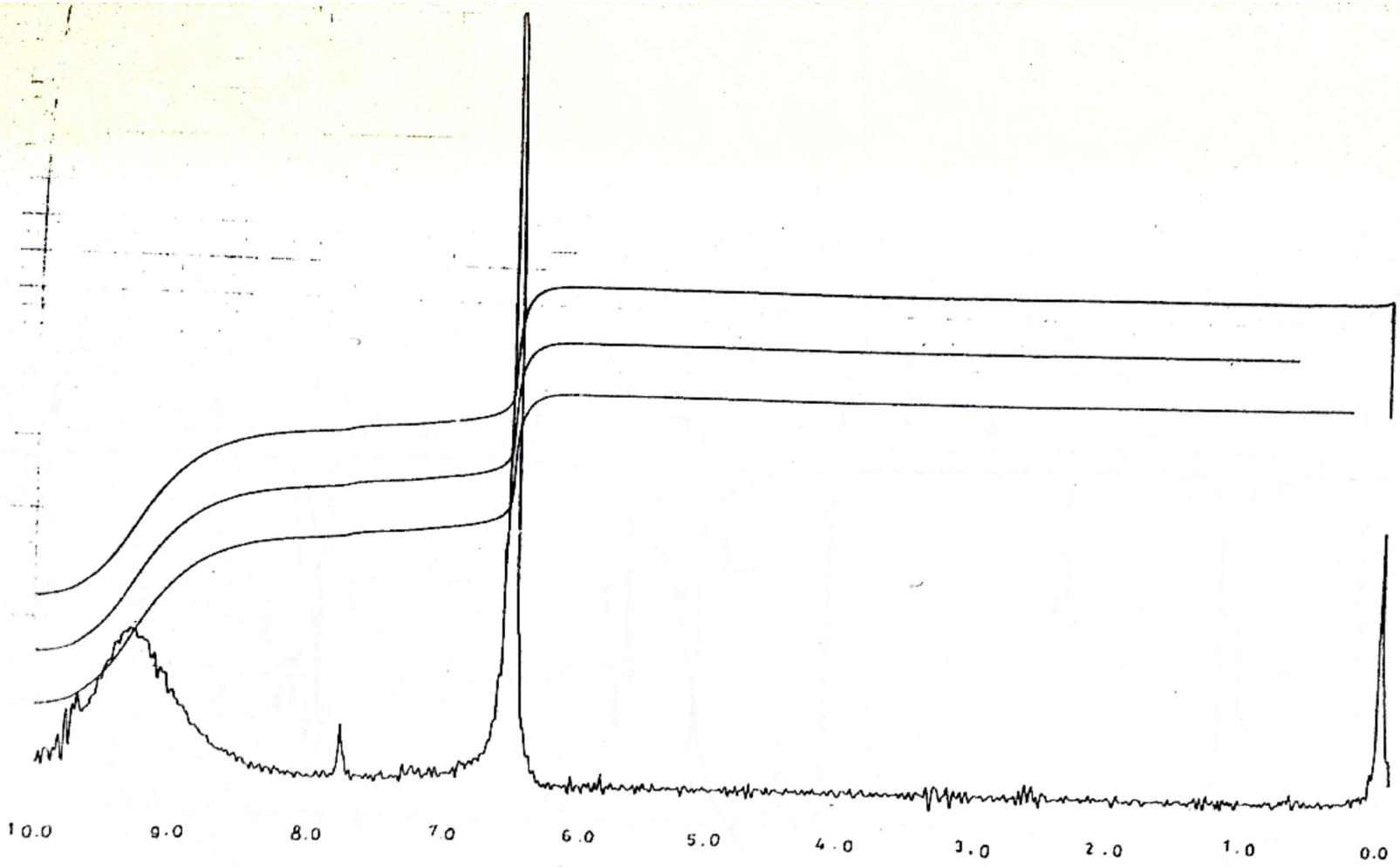

Fig. (1): Pmr spectrum of maleic anhydride (Internal standard).

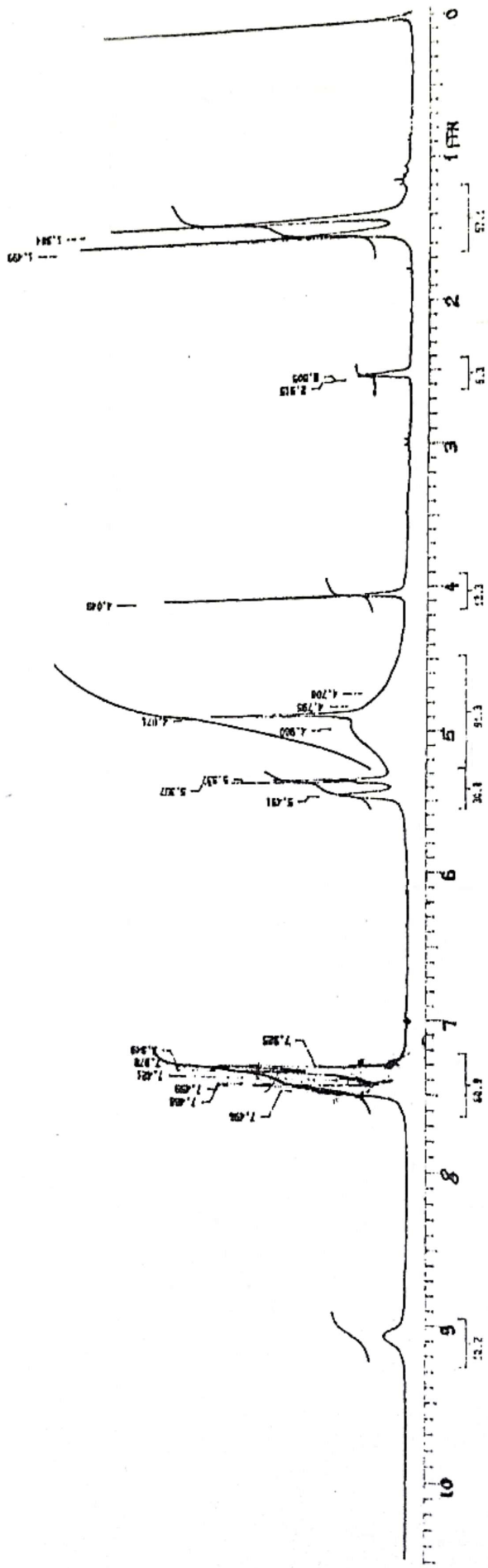

Fig. (2): Pmr spectrum of ampicillin.

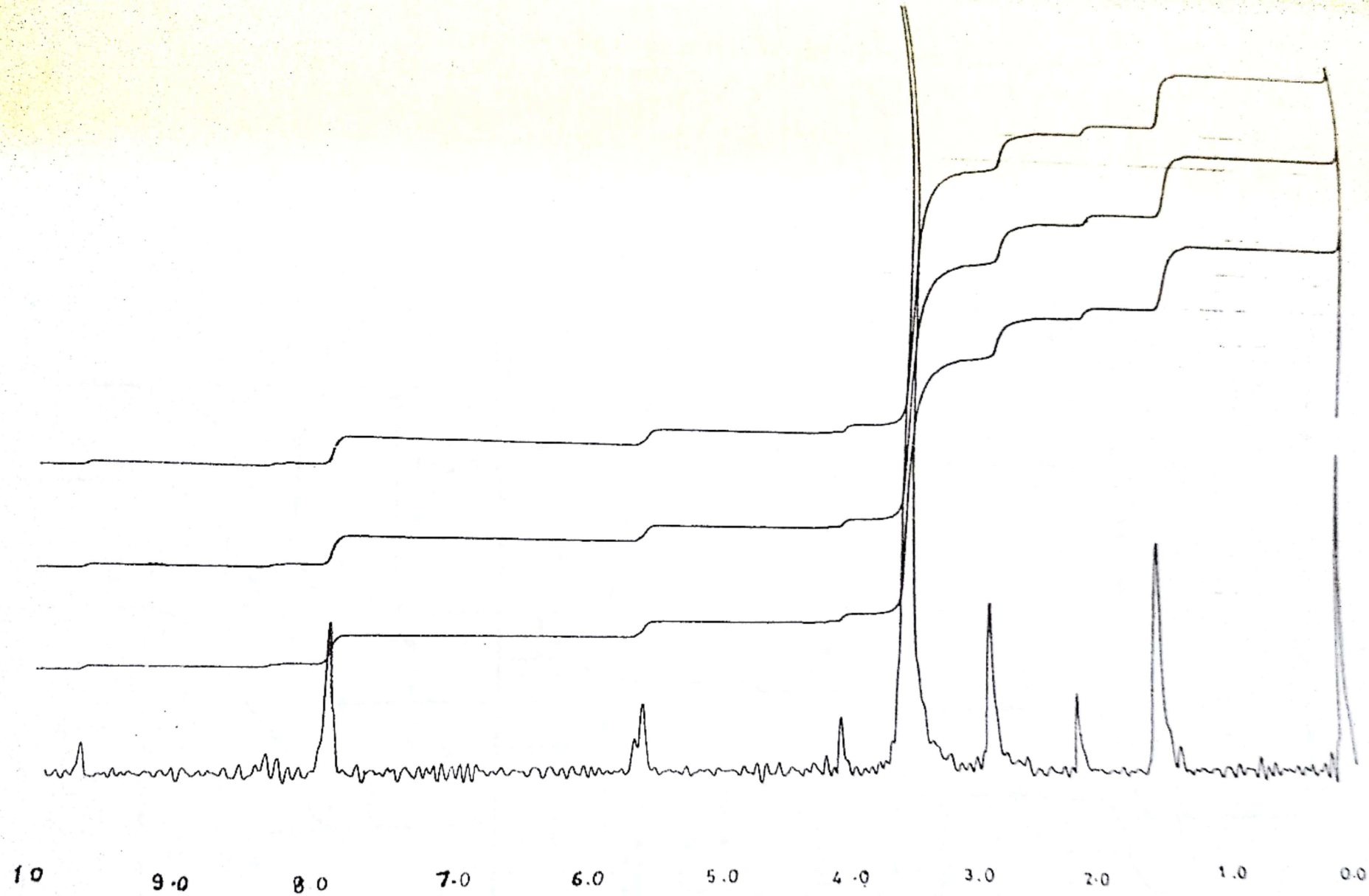

Fig. (3): Pmr spectrum of dicloxacillin.

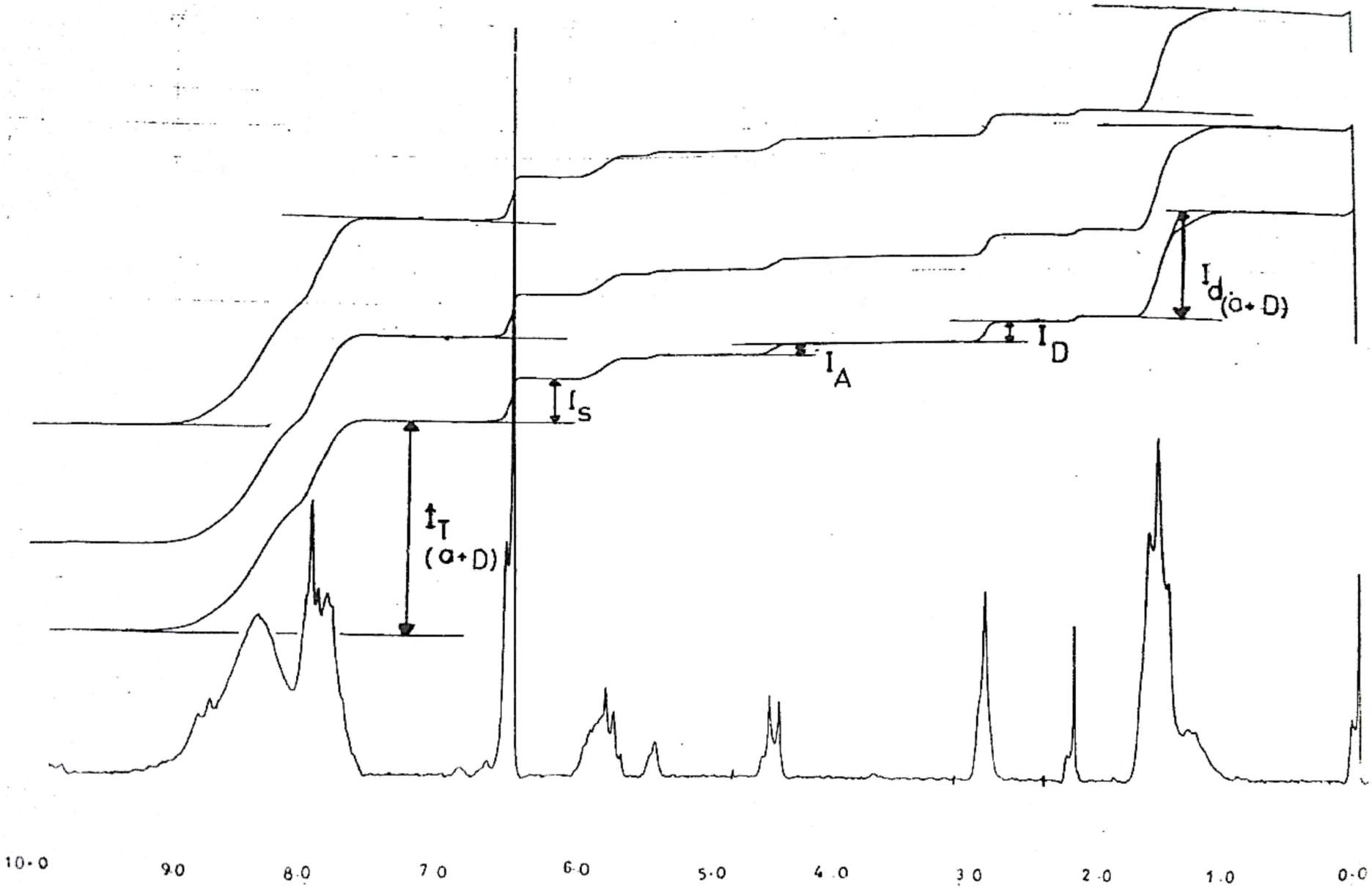

Fig. (4): Pmr spectrum of mixture of ampicillin and *dicloxacillin* in presence of internal standard maleic anhydride.

$Y = 0.24\ e^{0.39x}$

The logarithmic regression equation

$Y = 0.27 + 0.56 \ln x$

The power regression equation

$Y = 0.33\ X^{0.88}$

$\ln Y = \ln 0.33 + 0.88 \ln X.$

Table (3): Determination of dicloxacillin sodium using the proposed method and the iodometric method.

Dicloxacillin sodium taken mg	Dicloxacillin sodium found mg	% recovery iodometric * method (B.P.)	% recovery pmr method
26.6	26.48	99.1	100.29
24.8	25.95	99.2	104.65
41.8	40.05	98.7	95.83
88.4	87.48	98.9	98.96
88.4	87.40	99.0	98.87
		99.04±0.325	99.68±2.92
		V=0.292	V=8.55
		S.D=0.54	S.D=2.92
			T=4.03
			P= 12
			Fo=8.5x10-3

*(B.P.) British Pharmacoepeia (1973).

Table (4): Determination of ampicillin using the proposed method and official B.P method.

Ampicillin added	ampicillin found	B.P. method % recovery *	prroposed method % recovery
14.7	14.26	102.06	103.06
14.6	14.59	102.06	100.06
14.5	14.40	100.90	99.36
21.7	21.71	102.00	100.08
21.0	20.27	102.00	103.60
21.6	21.57		100.12
36.5	35.42		100.23
36.6	36.46		100.66
36.6	35.48		103.15
	101.588±0.715		101.15±1.63
	V=0.332		V=2.66
	S.D=0.715		S.D=1.63
			t=8.22

* British Pharmacoepeia (1973)

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تعيين مخلوط من الأمبيسلين والدايكلوكساسلين فى صورة جرعات بالرنين النووى المغناطيسى

ممدوح محمد فكرى متولى

قسم الكيمياء التحليلية – كلية الصيدلة – جامعة الزقازيق – مصر

إستخدمت المهارة الفنية للرنين المغناطيسى لتعيين كمية المشتقات البنسيلية مثل الأمبيسلين والدايكلوكساسلين واستعمال المالیك انهيدريد كمعيارى داخلى وقياس التجاوب التكاملى للأحادية التى تمثل أثنين من الهيدروجين التى تظهر على طول قدره ٦,٥٥ جزء من المليون ومقارنته لطول القمة لأثنين من مجموعتين الهيدروجين الأروماتية للأمبيسلين والدايكلوكساسلين التى تمثل ثمانية هيدروجين التى تظهر على صورة مضاعفة عند طول قدره ١,٥ جزء من المليون. وتم تعيين الأمبيسلين فى المخلوط بقياس التجاوب التكاملى لطول القمة المضاعفة لسبعة من الهيدروجين.

وجدت النسبة المئوية الأستردادية للأمبيسلين والدايكلوكساسلين محسوبة وتعطى نتائج جيدة مع متوسط نسبى استردادى لأمبيسلين قدره ١٠١,٥ + ١,٦٣ (المتوسط + معامل الانحياز). والدايكلوكساسلين قدره (٩٩,٦٨ + ٢,٩٢) ولكل من الأمبيسلين والدايكلوكساسلين (٩٩,٦٢ + ٥,٨٦) ومعادلة ارتداد للدايكلوكساسلين مساويه (ى = ٥,٤٤ × ١٠-٢ + ٠,٢٧).

بينما وجدت للأمبيسلين مساويه (ى = ٠,٩ × ١٠-٣ + ٠,١٢).