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Determination of Ampicillin and Cloxacillin Mixture by NMR

Moon-Hee Shin, Man-Ki Park, Chang-Hwa Yu and Jung-Kap Choi

Department of Pharmaceutics, College of Pharmacy, Seoul National University, Seoul 151, Korea

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Abstract□A simple, accurate and specific NMR procedure is described for the determination of ampicillin and cloxacillin mixtures in injection dosage form and capsules. The solvent was dimethylsulfoxde-d_6 and maleic acid was the internal standard. By integrating the peak at 2.68 ppm and 4.57 ppm, cloxacillin and ampicillin could be determined respectively. The relative proton ratio of ampicillin trihydrate and cloxacillin were 1.038 and 0.950. The coefficents of variation of ampicillin trihydrate and cloxacillin in a few commercial preparation were 1.55% (n=9), 2.69% (n=15).

Keywords□Ampicillin and cloxacillin mitxure-NMR procedure-Solvent; dimethylsulfoxide-d_6-Internal standard; maleic acid-Integration of the peak at 2.68 ppm and 4.57 ppm.

Recently considerable efforts have been devoted to the quantitative analysis of components in pharmaceutical mixtures by nuclear magnetic resonance (NMR) spectrometry.

Application of NMR spectrometry to antibiotics has been reported by William L. Wilson *et al.*[1]. In this report the NMR spectra of various commertially available penicillins and cephalosporins have been given.

A mixture of ampicillin and cloxacillin which provides a broad-spectrum antibiotic combination effective against penicillinase-producing staphylococci and most strains of gram-negative bacteria[2~3] was analyzed by NMR.

The analysis of this drug involves a variety of analytical techniques. Ampicillin and cloxacillin mixtures have been assayed by ion exchange chromatography[4], thin layer chromatography[5], colorimetry[6], ultraviolet (UV) spectrophotometry[7] and iodometry[8]. But these methods have been found to be time-consuming and complicated.

Ampicillin sodium (Amp · Na), ampicillin trihydrate (Amp · $3H_2O$) and cloxacillin (Clox) were assayed alone and mixed form by NMR spectrometry. The results were compared with those obtained by an UV spectrophotometry which has been reported by A. G. Davidson and J. B. Stenlake[7].

Analyzing the ampicillin and cloxacillin mixtures by NMR method was found to be simple, specific and accurate.

EXPERIMENTAL

Reagents

Standard : Amp. $3H_2O$ (assay: 84.22%, Chong Kun Dang Co.) Clox (assay: 875 mcg/mg, Chong Kun Dang Co.)

Internal standard : maleic acid (Wako

pure chemical industries)

Solvent : dimethylsulfoxided$_6$ for NMR spectroscopy (Merck)

Sample : Amp · Na & Clox 500 mg injections and Amp · $3H_2O$ & Clox 500mg, 250mg capsules from various commercial sources

Apparatus

NMR spectrometer : Perkin-Elmer R32 (90 MHz) All spectra were scanned at probe temperature of 35°C.

Micro-balance : Mattler type M-5

UV spectrophotometer: Pye Unicam SP 1750

Procedure

1) Standard procedure by NMR

Amp · Na, Amp · $3H_2O$, Clox were weighed about 40 mg accurately and were used alone or mixed form as a sample. The sample was transferred to a glass-stoppered tube and 25 mg of maleic acid (weighed accurately) was added as the internal standard. It was dissolved completely with 0.5 ml of DMSO-d_6. Then 0.4 ml of this solution was transferred to a NMR tube and one drop of tetramethylsilane (TMS) was added. The tube was placed in the NMR spectrometer and the spectrum was obtained.

All peak field positions were referred to TMS as 0 ppm on the delta scale. Each of the peak of interest was integrated at least five times. Then the amount of Amp and Clox was calculated individually as follows:

$$\text{mg Amp}=\frac{I_{Amp}\times \text{M. W.}_{Amp}/1}{I_{Mal}\times \text{M.W.}_{Mal}/2}\times \text{mg Mal}\times R_{PR}$$

$$\text{mg Clox}=\frac{I_{Clox}\times \text{M.W.}_{Clox}/3}{I_{Mal}\times \text{M.W.}_{Mal}/2}\times \text{mg Mal}\times R_{PR}$$

where I_{Amp} = integral value of the signal from Amp

I_{Clox} = integral value of the signal from Clox

I_{Mal} = integral value of the signal from maleic acid

M.W._{Amp} = molecular weight of Amp

M.W._{Clox} = molecular weight of Clox

M.W._{Mal} = molecular weight of maleic acid

R_{PR} = Relative proton ratio

$$R_{PR}\text{ of Amp}=\frac{I_{Amp}\times \text{M.W.}_{Amp}/1\times \text{mg Mal}}{I_{Mal}\times \text{M.W.}_{Mal}/2\times \text{mg Amp}}$$

$$R_{PR}\text{ of Clox}=\frac{I_{Clox}\times \text{M.W.}_{Clox}/3\times \text{mg Mal}}{I_{Mal}\times \text{M.W.}_{Mal}/2\times \text{mg Clox}}$$

2) Standard procedure by UV method

(1) Determination of ampicillin

The mixture was weighed correspond to 10 mg of Amp accurately and placed in 25 ml mass flask. The sample was dissolved and the flask was filled to 25 ml mark with pH 5 buffer solution. At the same time, the mixture was weighed same amount as above into 25 ml flask, dissolved and filled the flask to 25 ml with pH 9 buffer solution. The absorbance of the pH 5 solution of mixture was read against the pH 9 solution of the mixture at 268 nm. The concentration of Amp was determined by comparison with the absorbance of a standard solution of Amp.

(2) Determination of cloxacillin

The mixture was weighed correspond to 10 mg of Clox accurately and transferred to

25 ml mass flask. It was dissolved and filled the total volume to 25 ml with pH 5 buffer solution. The concentration of Clox was obtained by measurement of the absorbance at 275 nm of the mixture in pH 5 buffer solution read against a pH 5 buffer blank. The absorbance at 275 nm of a standard solution of Amp was also measured. The influence of Amp on the absorbance of sample at 275 nm was corrected. The corrected absorbance at 275 nm was propotional to the concentration of Clox.

RESULTS AND DISCUSSION

NMR Spectra of Amp. and Clox.

When 30 mg of the sample was taken, the signal to noise ratio was large enough to analyze. Therefore, in this experiment the sample amount was decided to 40 mg.

By NMR standard procedure, the spectra of Amp·Na, Amp·$3H_2O$ and Clox appeared as Fig. 1–3.

Fig. 4–5 represents the NMR spectra of Amp · Na and Clox, Amp · $3H_3O$ and Clox mixture.

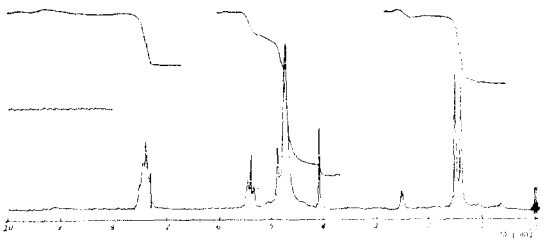

Fig. 2: The NMR spectrum of ampicillin. $3H_2O$.

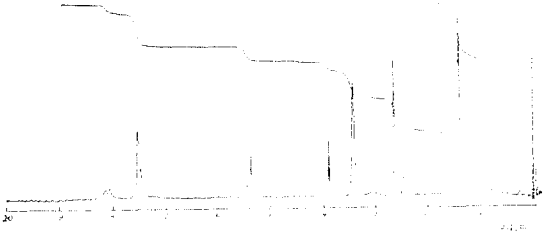

Fig. 3: The NMR spectrum of cloxacillin.

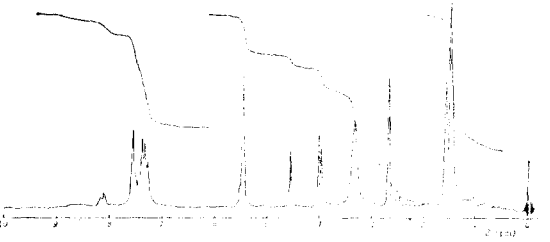

Fig. 4: The NMR spectrum of ampicillin · Na and cloxacillin mixture.

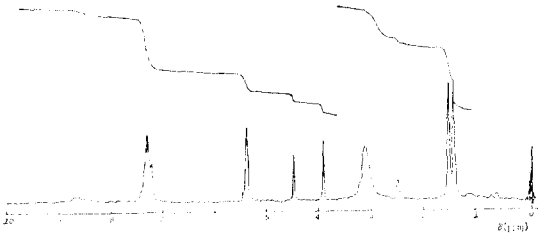

Fig. 1: The NMR spectrum of ampicillin · Na.

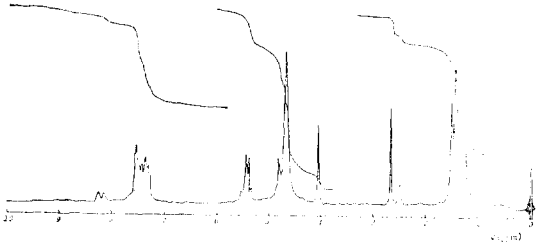

Fig. 5: The NMR spectrum of ampicillin · $3H_2O$ and cloxacillin. mixture.

As shown in Fig. 4, CH peak of Clox appeared at 2.68 ppm and CH peak of Amp · Na and Amp·$3H_2O$ at 4.57 ppm, specifically. By integrating these two peaks, Amp·Na and Clox could be determined easly without any interference.

In the NMR spectrum of Amp · $3H_2O$, Clox mixture (Fig. 5), it seem to be impossible to determine this mixture because the water peak and the CH peak of Amp · $3H_2O$ were overlapped.

The chemical shifts of Amp·Na, Amp·$3H_2O$ and Clox were appeared as Table I.

Selection of the Internal Standard.

The internal standard should not interact with sample and overlap with other peaks. It should freely dissolve in solvent and shows a sharp and single peak.

On this experiment, maleic acid which presents a sharp and single peak at 6.1–6.3 ppm found to be the most satisfied internal standard.

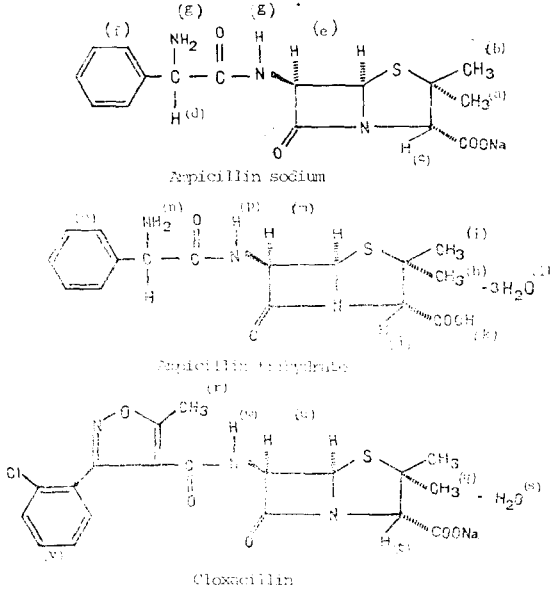

Table I: The chemical shifts of ampicillin · Na, ampicillin · $3H_2O$ and cloxacillin.

Amp · Na	Amp · $3H_2O$	Clox
a 1.47	h 1.40	q 1.46
b 1.58	i 1.50	r 2.70
c 3.98	j 4.08	s 3.52
d 4.50	k 4.90	t 3.95
e 5.46	l 4.96	u 5.46
f 7.37	m 5.40	5.52
g 8.70	n 6.95	v 7.59
	o 7.41	w 8.16
	p ~9	

Addition Effect of Maleic Acid as the Internal Standard

Adding 10 mg of maleic acid in the mixture, the water peak of Clox disppeared. And the integration of maleic acid was difficult because the maleic acid peak was overlapped with a broad peak (Fig. 6).

Then the amount of maleic acid was increased 15,20,25 mg in the mixture separately. When 25 mg of maleic acid was added, the maleic acid peak was separated completely so that the integration of maleic acid was possible (Fig. 7).

So in this experiment, the amount of maleic acid was decided to 25 mg.

In Fig. 7, it was noticeable that the water peak of Clox and COOH peak of maleic acid were disappeared and a very broad peak was appeared at 8–10 ppm.

Interaction between Maleic Acid and Water

When water was added in maleic acid from 2 μl to 10 μl gradually, an interaction was found between COOH of maleic acid and water (Fig. 8).

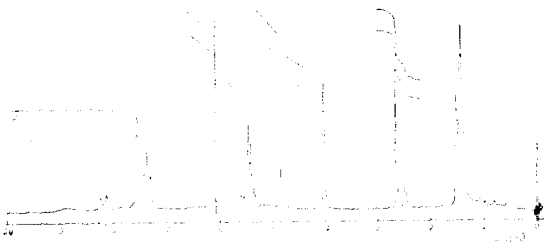

Fig. 6: The NMR spectrum of maleic acid as an internal standard (added 10 mg).

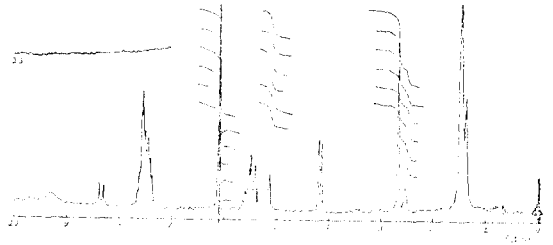

Fig. 7: The NMR spectrum of maleic acid as an internal standard (added 25mg).

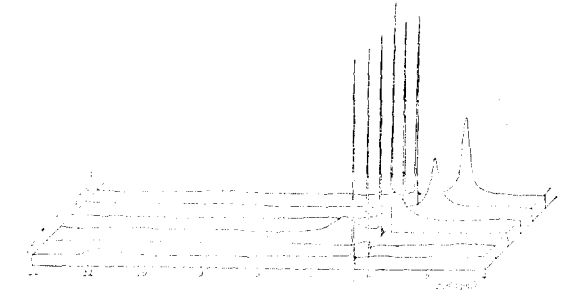

Fig. 8: Interaction of maleic acid and water.

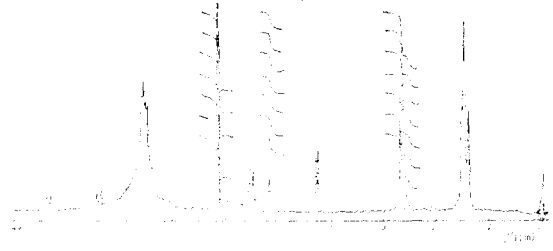

Fig. 9: The NMR spectrum of ampicillin · $3H_2O$ cloxacillin mixture & maleic acid.

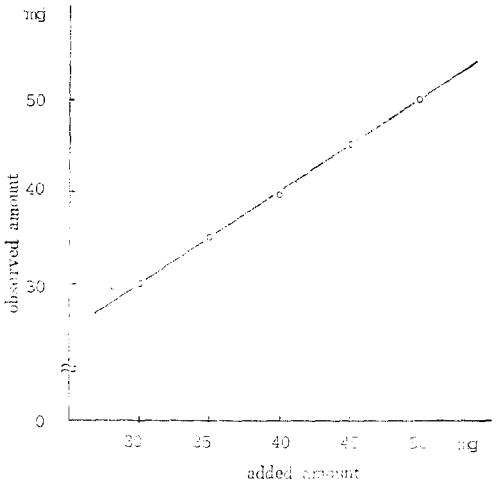

Fig. 10: Ampicillin · $3H_2O$ calibration curve by NMR

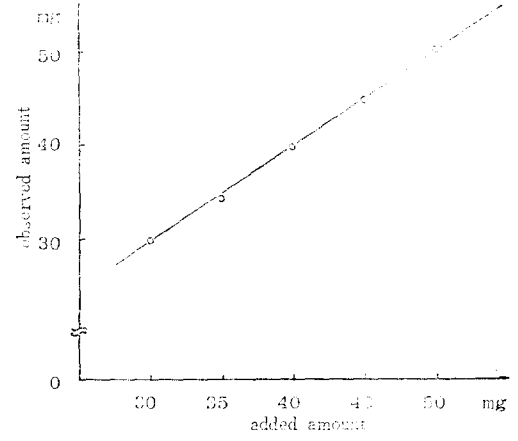

Fig. 11: Cloxacillin calibration curve by NMR.

As a result of this interaction, water peak was shifted to the low field (water peak ordinarily appears at 4.7 ppm). From this fact, the broad peak which was overlapped with maleic acid in Fig. 6 was found to be the crystal water of Clox.

Because the maleic acid moved the water peak, Amp · $3H_2O$ & Clox mixture was possible to determine which seemed to be

Table II: Determination of Amp and Clox standard mixtures by NMR.

Maleic acid added mg	Amp · Na			Clox			Amp · 3H O		
	added mg	found mg	recovery %	added mg	found mg	recovery %	added mg	found mg	recovery %
25.195	40.320	40.493	100.43	—	—	—	—	—	—
25.260	40.000	39.699	99.25	—	—	—	—	—	—
25.630	40.460	41.244	101.94	—	—	—	—	—	—
25.115	—	—	—	40.755	41.319	102.35	—	—	—
25.300	—	—	—	41.230	42.698	103.56	—	—	—
25.025	—	—	—	39.360	40.974	104.10	—	—	—
25.170	—	—	—	—	—	—	40.335	41.752	103.51
25.035	—	—	—	—	—	—	39.960	41.247	103.22
25.030	—	—	—	—	—	—	41.445	42.668	102.95
24.970	39.575	39.738	100.41	40.755	41.829	102.64	—	—	—
25.350	40.820	40.160	98.38	39.910	40.064	100.39	—	—	—
25.160	40.100	39.740	99.10	41.600	41.235	99.12	—	—	—
25.085	—	—	—	39.825	40.460	101.59	39.880	39.893	100.03
25.310	—	—	—	39.945	40.913	102.42	40.125	40.300	100.44
25.330	—	—	—	40.320	40.601	100.70	40.120	39.721	99.00
	M.V.=99.92			M.V.=101.87			M.V.=101.53		
	C. V.= 1.16%			C. V.=1.47%			C. V.=1.74%		

M. V.= mean value

C. V. = coefficent of variation

impossible (Fig. 9).

Calibration Curve

Amp and Clox standard were calibrated respectively by NMR method in the range of 30–50mg per 0.5ml (Fig. 10–11).

Good linearities were found both in Amp and in Clox calibration curve. Therefore, the quantitative analysis of Amp and Clox

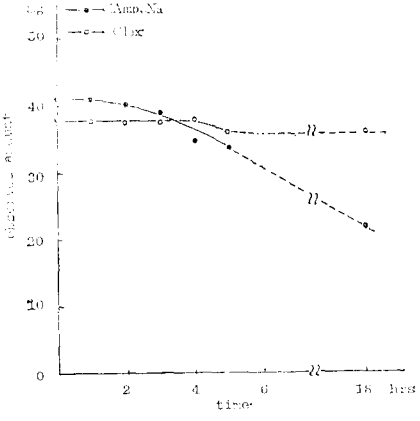

Fig. 12: Stability of ampicillin Na · and cloxacillin. mixture.

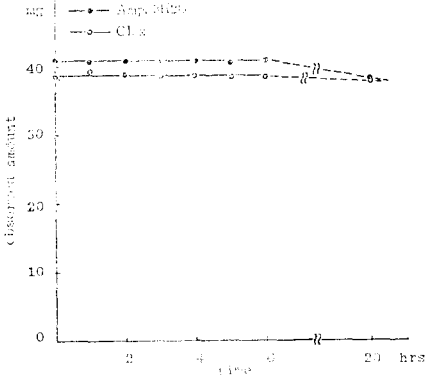

Fig. 13. Stability of ampicillin · $3H_2O$ and cloxacillin mixture.

Table III: Determination of ampicillin and cloxacillin in standard mixtures by UV.

Amp			Clox		
added mg	found mg	recovery %	added mg	found mg	recovery %
9.97	9.98	98.50	—	—	—
9.96	9.82	98.60	—	—	—
9.98	9.91	99.30	—	—	—
9.96	9.82	98.59	—	—	—
—	—	—	10.03	10.08	100.53
—	—	—	10.01	9.97	99.60
—	—	—	10.07	10.00	99.30
—	—	—	10.00	9.92	99.20
9.97	9.82	98.50	10.03	10.08	100.53
9.96	9.82	98.59	10.01	9.97	99.60
9.98	9.91	99.30	10.07	10.00	99.30
9.96	9.82	99.89	10.00	9.92	99.20
M. V.=98.75 C. V.=0.33%			M. V.=99.66 C. V. =0.53%		

M. V.=mean value C. V.=coefficent of variation

was possible in this range.

Also relative proton ratio was determined by these standard treatment. Relative proton ratio of Amp was 1.038 and that of Clox was 0.950.

Determination of Standard Mixture

Amp · Na, Amp · $3H_2O$, Clox standard alone and mixtures were assayed by NMR method (Table Ⅱ, and by UV method (Table Ⅲ).

NMR method was more accurate than UV method but less precise than UV method.

Stability of the Sample Solution

After assaying the mixture, the stability was observed for 18–20 hours. As shown in Fig. 12, Amp · Na was decreased 2.3% after 2 hrs, 50 % after 18 hrs.

In Fig. 13, Amp · $3H_2O$ and Clox were still stable after 6 hrs. and only 6.9 % of Amp·$3H_2O$ and 1.6 % of Clox were decreased after 20 hrs.

Therefore, if the whole procedure would be finished within 2 hrs, the error could be minimized.

Determination in Commercial Preparations

Two lots of Amp · Na & Clox mixtures in vials and three lots of Amp · $3H_2O$ & Clox mixtures in capsules were assayed by NMR method. The relative contents to the declared strength of each compound were calculated as follows.

$$\text{Amp } \% = \frac{A_{SA\ mg}}{A_{ST\ mg}} \times SA \times \frac{\text{filled amount}}{DS} \times 100$$

$$\text{Clox } \% = \frac{C_{SA\ mg}}{C_{ST\ mg}} \times SC \times \frac{\text{filled amount}}{DS} \times 100$$

where $A_{SA\ mg}$=Amp sample amount found by NMR method

$A_{ST\ mg}$=Amp standard amount found by NMR method

$C_{SA\ mg}$=Clox sample amount found by NMR method

$C_{ST\ mg}$=Clox standard amount

Table IV: Determination of ampicillin & cloxacillin in commertial mixtrues by NMR.

Maleic acid mg	Sample filled in vial or cap. mg	Taken sample mg	found by NMR (mg)			recovery %		
			Amp · Na	Amp · $3H_2O$	Clox	Amp · Na	Amp · $3H_2O$	Clox
25.090	582.080	80.740	46.429	—	40.394		—	101.92
25.700	576.740	80.082	42.019	—	40.853		—	102.96
25.070	573.500	80.095	41.528	—	40.046		—	100.36
25.535	565.510	79.855	39.433	—	42.645		—	105.70
25.165	555.250	80.265	41.588	—	40.695		—	98.53
25.535	571.785	80.310	40.203	—	42.341		—	102.52
25.025	591.825	80.035	—	40.445	40.432	—	100.40	104.29
25.610	604.720	80.500	—	40.640	40.511	—	102.85	106.51
25.415	616.475	80.485	—	40.479	37.956	—	104.45	101.75
25.375	610.750	79.990	—	39.074	40.334	—	100.51	107.78
25.275	608.610	80.565	—	39.663	40.649	—	100.94	107.48
25.130	603.760	80.295	—	38.960	39.713	—	98.69	104.51
25.225	299.890	80.230	—	40.033	40.871	—	100.82	106.94
26.015	310.905	80.595	—	38.598	40.035	—	100.32	108.11
25.245	301.580	80.760	—	40.591	39.142	—	101.95	103.02
							M. V.=101.21	104.16
							C. V.= 1.55%	2.69%

found by NMR method
TA =strength of Amp standard
TC =strength of Clox standard
DT =declared strength

The result appeared as Table Ⅳ

The content of Amp. Na could not be determined because it was difficult to obtain the standard of Amp · Na in this experiment.

CONCLUSION

1. In NMR spectrum of Amp · Na & Clox mixture, the peak at 2.58 ppm represented Clox and that at 4.57 ppm represented Amp specifically.

By integrating these peaks, Amp and Clox could be determined. Maleic acid was used as the internal standard.

2. Amp · $3H_2O$ & Clox mixtures seemed to be impossible to determine because CH peak and water peak of Amp·$3H_2O$ were overlapped But maleic acid interacted with water of Amp. $3H_2O$ and shifted the water peak to separate the CH peak. Therefore, it was possible to determine the Amp. $3H_2O$ mixture.

3. Because the calibration curve represented a good linearity, it was sure that observed amount by NMR method was proportional to the standard amount.

The relative proton ratio of Amp · $3H_2O$ and Clox was 1.038 and 0.950 respectively

4. The Clox and Amp · $3H_2O$ were stable but the Amp · Na was rather unstable. The whole NMR procedure had to be finished within 2 hrs.

5. The coefficients of variation of Amp·$3H_2O$ and Clox in various commercial preparations were 1.55 % (n=9) and 2.69 % (n=15).

6. Determination by NMR method was simple, specific than UV method, and accurate. It took only 15 mins to determine a sample.

This determination of Amp and Clox by NMR could be used as a base of micro-analysis by using time averaging computer and drug monitorring in biological material.

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