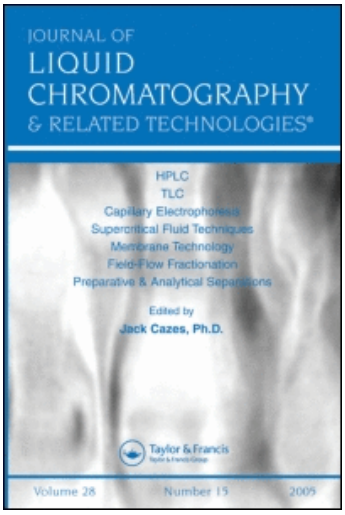

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Simultaneous Determination of Ampicillin and Dicloxacillin in Pharmaceutical Formulations by High-Performance Liquid Chromatography

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SIMULTANEOUS DETERMINATION OF AMPICILLIN AND DICLOXACILLIN IN PHARMACEUTICAL FORMULATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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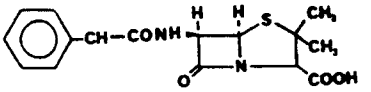
ABSTRACT

A rapid liquid chromatographic procedure for simple determination of each of ampicillin and dicloxacillin in bulk forms, their binary admixtures and in dosage forms is illustrated. The assay method has been adopted for simultaneous quantification of both penicillins in the presence of their degradation products. The best HPLC-resolution could be achieved on a reversed-phase, LiChrospher 100 RP-18 (5 μm), column by using a mobile phase containing acetonitrile + acetic acid (1%, aqueous) (39:61, v/v) isocratically at a rate of 2 ml.min^{-1} with UV-detection at 240 nm. Recovery testing of varying masses of each individual penicillin added to dosage forms was found to be fairly satisfactory.

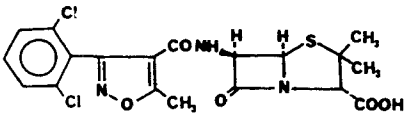
INTRODUCTION

Synergisms due to certain penicillins admixtures have been demonstrated against clinical isolates of some β-lactamase-producing and non-producing bacteria (1,2). Penicillins combinations exhibit more broad spectra and usually prescribed for infections induced by β-lactamase-producing strains. Synergy between ampicillin [I] and dicloxacillin [II] has been evaluated, so; several combinations containing them are now available on drug market.

The British Pharmacopoeia (BP 1988) (3) describes a mercurometric method after hydrolysing the β-lactame ring or complexation with an imidazole-mercury reagent followed by measuring the absorbance at 325 nm for determination of ampicillin in pure form and in



(I)



(II)

pharmaceutical preparations. While, the United States Pharmacopeia (USP 1990) recommends the high performance liquid chromatography and iodometric titrimetry for determination of ampicillin. Dicloxacillin is only official in the USPXXII (USP 1990) (4) which describes an HPLC-procedure for its determination in bulk material and pharmaceutical preparations. The difficulty arises when a simultaneous quantification of both penicillins is required.

Reversed-phase liquid chromatography, RP-HPLC (5) and reversed-phase thin-layer chromatography, RP-TLC (6) have been described only for separation and identification of several penicillins, including ampicillin and dicloxacillin, but not for their quantification. Various HPLC-methods have been reported for the determination of β-lactame antibiotics, individually (7-12). Recently, HPLC-methods with derivative UV-detection (13) and normal-mode UV-detection (14) have been investigated for determination of amoxycillin and dicloxacillin in capsules. A potentiometric procedure for the contemporaneous determination of ampicillin and dicloxacillin as such, in their binary mixtures, and in capsules, has been described (15).

It appears to be of high value to develop rapid and accurate assay methods for simultaneous estimation of ampicillin and dicloxacillin. The versatility of HPLC in antibiotics assay

is well discussed (16). So, the present work describes a simple liquid chromatographic-method for determination of the intact individual antibiotics in their binary admixtures and in some dosage forms. The stability-indicating characteristics, i.e. determination of the intact penicillins in the presence of their degradation products were investigated.

EXPERIMENTAL

Materials

Ampicillin trihydrate-WHO reference, assessed purity 99.4% (BP 1988), water content 12.5%- and dicloxacillin sodium monohydrate - claimed purity 99.5%, water content 3.9%, Gruppo Lepetit S.p.A., Milan-Italy - were utilized without further treatments. Capsules and powders for suspensions containing ampicillin and dicloxacillin were purchased randomly from local pharmacies. The penicillin contents in all investigated preparations were calculated as the free anhydrous bases. The antibiotics and their capsules were kept in a dry cool place in tightly closed moisture-proof containers.

Acetonitrile, HiPerSolv (HPLC-grade), pure glacial acetic acid (AnalaR™), BDH Chemicals, Poole-U.K., and all-glass bidistilled water was used to prepare the mobile phase.

Instrument

Shimadzu LC-10 AD liquid chromatograph was attached to SPD-10A tunable UV-detector, CTO-10A column oven controller, DGU-3A mechanical degasser, and C-R4A Chromatopac data unit, Shimadzu Corp., Analytical Instruments Division, Kyoto-Japan. Fixed loop injector (Rhydone, 20-μl) was utilized to carry the samples onto the column.

Analytical Procedure

Chromatographic conditions

The separation was performed isocratically at a rate of 2 ml.min^{-1} on a reversed-phase [LiChrospher RP-18 (5 μm) Hibar prepacked column (12.5 cm × 4 mm ϕ), E. Merck, Darmstadt-

F.R.G.] at ambient temperature by using a mobile phase consisting of CH_3CN + 1% aq. CH_3COOH (39:61, v/v). The detection wavelength was set at 240 nm (AUFS = 1×10^{-3}).

Calibration graphs

Working standard solutions containing 50-200 $\mu g.ml^{-1}$ of each penicillin were prepared by taking 0.5-2 ml from the stock solutions (1 $mg.ml^{-1}$) of one penicillin in the mobile phase into separate 10-ml volumetric flasks and each was diluted with the mobile phase to the volume. Triplicate injections of each solution were done to get the standard plot of each antibiotic. Linear regression equations were computed for each penicillin:

$$Y = -0.07 + 0.089\ C(amp.)\ (r = 0.998)$$

$$Y = -0.031 + 0.327\ C(diclox)\ (r = 0.999)$$

where; Y; the area count ($\times 10^{-5}$) of the penicillin, C; the concentration ($\mu g.ml^{-1}$), and r; the correlation coefficient.

Alternatively, triplicate injections of the samples were done with similar standard working solutions in order to enable reliable sample/standard matchings. Calculation of drug contents was achieved by adopting the following formula:

$$C\ (mg.capsule^{-1}) = \frac{A_\mu D C(\%)\ W_1}{A_s W_2}$$

where, A_u and A_s are the areas for the ampicillin in sample (unknown) and standard, respectively, D is the dilution factor, W_1 and W_2 are the average weight (mg) of a capsule or 1-g powder for suspension and the taken weight (mg), in order, C(%) is the concentration percent of standard solution in the final dilution.

Recovery Testing

Spicking with equimasses of each individual reference penicillin was undertaken by adding varying aliquots to admixture solutions (25-100 $\mu g.ml^{-1}$) in the mobile phase. To test the degree

of recovery of the added amounts of each drug, triplicate injections of the solution mixtures were carried out. The reproducibility was evaluated by 12 consecutive injections of a standard solution equivalent to full amounts of each component drug based on its theoretical quantities.

RESULTS AND DISCUSSION

On adopting the method of the British Pharmacopoeia (BP-1988) (3) recommended for determination of ampicillin on dosage formulations specimens containing ampicillin and dicloxacillin combination, the reaction products of imidazole-mercury reagent with dicloxacillin show overlapping absorption at the wavelength specified for ampicillin. On the other hand, binary mixtures of both penicillins exhibit considerable UV-band overlap, which makes the direct UV-spectrophotometric determination of one penicillin in the presence of the other remains quite unrealizable.

The potentiometric titrimetry (15), described for simultaneous determination of ampicillin and dicloxacillin in capsules, has been tested for its applicability on degraded penicillin, where poor stability-indication was proved. The method depends on the fact that the amino group of ampicillin and the carboxylate group of dicloxacillin are differentially protonated, but most of the penicillin degradates still contain such functional grouping and behave on protonation likely as the intact penicillin.

Depending on conditions, the degradative pathway and products, i.e. degradates, are widely different. The main penicillins' degradates are penicilloic, penacilloic, penicillenic, penilloic and penillic acids (17). Detection of any penicillin degradation is of special interest in view of the implications of possible degradates in allergic responses to penicillin therapy (17). Analytical methods with significant stability-indicating characteristic are always needed for detection and determination of drug degradation as well as for quantification of the intact drug in presence of its prime and minor degradates.

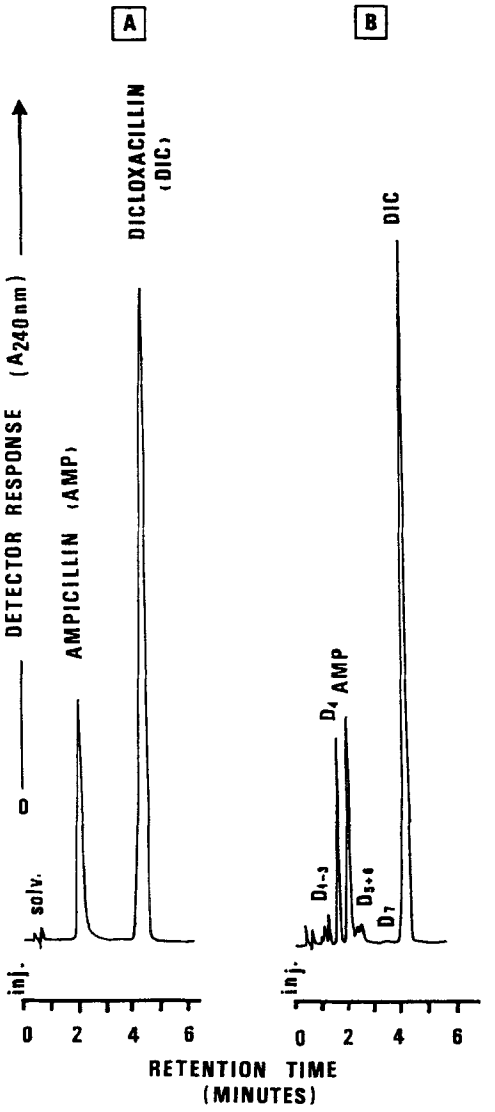


Figure 1: HPLC-Separation of (A) admixture of refrence ampicillin & dicloxacillin, and (B) same sample degraded at room temperature (22°C) for 24 hours; D means degradates.

HPLC has achieved pronounced advantages over some other techniques in being the most stability-indicating. The liquid chromatographic procedure herein investigated thus allows the simultaneous determination of ampicillin and dicloxacillin in admixtures even in the presence of their products of degradation. A reversed-phase column, namely, LiChrospher 100 C18 (5 μm), has revealed validity on utilizing the isocratic elution at 2 $ml.min^{-1}$ rate of flow with a polar mobile phase composed of acetronitrile and 1% aq. acetic acid (39:61, v/v). Symmetric peaks were obtained for both penicillins, the tailing factors, i.e. asymmetry factors, were 1.12 and 1.02 for ampicillin and dicloxacillin, respectively. The relative standard deviation (SDrel.) for twelve replicates was $\leq$ 2.0%. Figure 1 demonstrates the HPLC-resolution of both penicillins before and after degradation (24 hrs at 22°C). All the penicillin degradates $D_{1\text{-}7}$ were resolved quite away from the peaks of the two intact drug substances. Several chemicals, such as clavulanic acid, amoxycillin, ... etc., have been tried to serve as an internal standard but they were eluted either with one of the penicillins or near the retention times of the degradates. Fixed-loop injections as well as standard/sample matching give always consistent results. The mean deviations in case of ampicillin were relatively higher than that of dicloxacillin. This may be attributed to the slightly higher absorption coefficient (ϵ) of dicloxacillin at the specified wavelength for drug detection (240 nm). The selected chromatographic conditions seemed to be the best for indicating the penicillins stability.

Table 1 collects the obtained results of assay, recovery and reproducibility testing of the proposed chromatographic method of analysis. The described method is rapid , so, the two penicillins and all degradates leave the column completely after about 5 minutes. No sample clean-up or extra sample preparation are needed. The described HPLC-method has proved its advantage in the capability of finding the accurate contents of each drug substance in the presence of its degradates. The drug degradation was undertaken in solution either by one-day stay at room temperature (~22°C) or heating for 20 minutes. No justification of the decay products has been

Table 1: HPLC-Analysis (assay and recovery) of ampicillin and dicloxacillin in capsules.

Pharmaceutical Formulations	Analysis*	Penicillin content (%)	
		Ampicillin	Dicloxacillin
Cloxapen-250™ Capsules[a]	A	100.4 ± 1.56 (5)	100.5 ± 0.22 (5)
	R**	99.7 ± 0.50 (4)	99.9 ± 0.38 (6)
Cloxapen-500™ Capsules[a]	A	101.2 ± 2.34 (4)	100.3 ± 0.58 (4)
	R**	100.8 ± 0.50 (4)	100.2 ± 0.86 (4)
Diclopen™ Capsules[b]	A	102.4 ± 0.60 (5)	101.0 ± 0.68 (5)
	R**	101.1 ± 0.83 (4)	100.0 ± 0.46 (4)
Dipenacid™ Suspension[c]	A	99.6 ± 2.10 (6)	99.4 ± 0.94 (6)
	R**	100.4 ± 0.94 (4)	100.1 ± 0.63 (4)

*X ± CV (n); n : the average mean of at least 3 determinations.
A; assay R; recovery
**recovery of added mass (50%) of the named penicillin.
a. Cloxapen™ (250 & 500) capsules are products of each capsule contains equal amount (125 or 250 mg) of each penicillin (anhydrous base).
b. Diclopen™ capsules is a product of Kahira Pharm. & Chem. Ind. Co., Cairo-Egypt.
c. Dipenacid powder for oral suspension, dissoluted 5 ml contain 250 mg of each penicillin, is manufactured by CID Co., Giza-Egypt.

done in this study. Moreover, the recovery testing of the investigated method indicates good accuracy and precision. Excellent reproducibilities have been observed for different replicates of both penicillins as reflected in the relative low coefficient of variations.

It can be concluded that the proposed method for simultaneous quantification of ampicillin and dicloxacillin in their admixtures and/or pharmaceutical preparations is simple, rapid, quite accurate and stability-indicating in addition to its high precision and confidence.

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