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Stability-indicating analysis of isoxazolyl penicillins using dual wavelength high-performance liquid chromatography

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Abstract

Stability-indicating high-performance liquid chromatography analytical procedures were developed for specific determination of four isoxazolyl penicillins during degradation under neutral and accelerated acid/alkali conditions. The chromatographic conditions were set so that the drug peak was well separated from the peaks of the degradation products. Peak homogeneity of the resolving drug peak was assessed by the shape of the ratio chromatogram. Good and reproducible separations were achieved on a reversed-phase column using a mobile phase consisting of acetonitrile and a solution of 20 m*M* potassium dihydrogen phosphate plus 10 m*M* tetramethylammonium chloride in water (adjusted to pH 5). Optimal separations for all four drugs were achieved within the range of 15–21% organic modifier in the mobile phase. The detection wavelengths were 220 nm and 240 nm. The stability-indicating nature of the methods was confirmed by the linearity of the pseudo-first order plots. The utility of dual-wavelength chromatography in establishment of stability-indicating assays is highlighted. © 1998 Elsevier Science B.V.

Keywords: Isoxazolyl penicillins; Penicillins

1. Introduction

The setting up of stability assays of penicillins is complex. These drugs are known to yield a formidable array of degradation products, which range from the expected opened β-lactam ring products (e.g. penicilloic acid), various rearrangement products derived from the nucleus as well as the side chain, and the products of polymerization [1–3]. The identification/characterisation of large numbers of degradation products, both major and minor, is a laborious exercise and to date has been attempted for only a small number of these drugs.

Among the isoxazolyl penicillin group, comprising four drugs oxacillin (OLX), cloxacillin (CLX), dicloxacillin (DLX) and flucloxacillin (FLX), HPLC methods for specific determination of the drug in the presence of degradation products are reported in the literature mainly for CLX [1–4] and DLX [5,6]. To our knowledge there is no report on specific HPLC analysis of OLX and FLX. Even among the reported methods for CLX and DLX, most of them suggest conditions for the determination of the drug in the presence of a few isolated major degradation products and do not account for the other minor/major products formed during decomposition.

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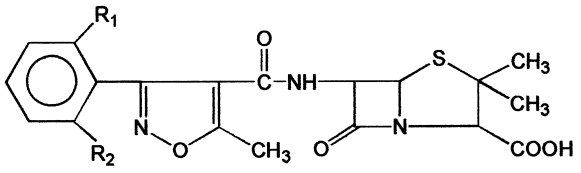

R_1	R_2	Drug
H	H	Oxacillin (OLX)
Cl	H	Cloxacillin (CLX)
Cl	Cl	Dicloxacillin DLX)
F	Cl	Flucloxacillin (FLX)

Fig. 1. Chemical structures of isoxazolyl penicillins.

The recent regulatory guidelines [7] strongly suggest that for validation of analytical methods for the stability program, studies should be conducted on the drug substance under accelerated and stress conditions. The purpose of the present study, therefore, was to establish assay conditions under which the four isoxazolyl penicillins (for structures see Fig. 1) could be specifically analysed in the presence of degradation products formed during the actual decomposition at various pH values.

2. Experimental

2.1. Materials and reagents

Oxacillin, cloxacillin, dicloxacillin and flucloxacillin were supplied gratis by Beecham, UK and were used as such without further purification. HPLC grade solvents (Merck, Bombay, India) and fresh triply distilled water obtained from an all-glass still (Scientronic, New Delhi, India) were used in all studies. Buffer materials and all other chemicals were of analytical-reagent grade.

2.2. Preparation of reaction solutions

The reaction solutions were prepared by dissolving the drugs at a concentration of 100 $\mu g\ ml^{-1}$ in buffer of required pH (2.0, 7.0 or 9.2) and adjusted to an ionic strength 0.2 using sodium chloride. The pH were measured on a Metrohm 654 research pH meter (Metrohm, Herisau, Switzerland) calibrated at the temperature of study using standard buffers [8].

2.3. Degradation studies

Solutions were incubated in stoppered glass tubes in a precision water bath (D8, Haake, Germany). The temperatures used for basic, acidic, and neutral conditions were 35°C±0.1, 50°C±0.1 and 80°C±0.2, respectively. Aliquots were withdrawn at appropriate time intervals and subjected to analysis. The studies were conducted in triplicate.

2.4. Separation studies

The separations were carried out on a Shimadzu LC-10A series chromatograph equipped with two LC-10AS solvent delivery units, an SPD-10A dual-wavelength UV–vis detector, a C-R7A Chromatopac data processor fitted with an additional channel board (all Shimadzu, Kyoto, Japan) and a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 20-μl fixed loop. During the studies, the detector was lit in the dual-wavelength mode. The detector collected the data in this mode by sequential scanning of grating [9]. The wavelength and absorbance values are monitored independently at each wavelength and by virtue of it, the detector either allows simultaneous recording of chromatogram for both wavelengths on the two channels of Chromatopac data processor, or it lets simultaneous recording of chromatogram at one wavelength on one channel and ratio chromatogram of two wavelengths on the other. The data processor in our studies was set in the latter condition.

Separation of a drug from degradation products was achieved on a Phenomenex Resolve C_{18} (5 μm) 300×3.9 mm column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and a solution of 20 m*M* potassium dihydrogen phosphate and 10 m*M* tetramethylammonium chloride (TMA) in water (pH 5.0). It was filtered through a 0.45-μm membrane and degassed at reduced pressure in an ultrasonic bath (3210E-DTH, Bransons, CT, USA). The flow-rate in all the cases was kept at 1 $ml\ min^{-1}$. The two detection wavelengths were

220 nm and 240 nm. The peak area was used as a measure of drug quantity.

The response of the detector was found to be linear for all drugs in the concentration range of 5–150 $\mu g\,ml^{-1}$ ($r^2>0.99$). The reproducibility was verified by six replicate injections of each drug (C.V. $\ll 1\%$) at three different concentrations, 5, 75 and 150 $\mu g\,ml^{-1}$.

3. Results and discussion

3.1. Chromatographic separation of drug from products

Lauriault et al. reported the determination of CLX [4] and DLX [6] in the presence of respective penilloic, penicilloic and penicillanic acids, which were synthesised or isolated in their laboratory. The separations were achieved on a reversed-phase column using acetonitrile–phosphate buffer (pH 4.5) as the mobile phase. The ratio of organic modifier to buffer was 20:80 for CLX and 25:75 for DLX.

This reported method was tried initially for the specific determination of all four drugs from their reaction mixtures in neutral and accelerated acid/alkaline conditions. However, the resolution of the drug peak from other peaks was not satisfactory, especially during degradation in acidic conditions where multiple peaks appeared in the chromatograms.

Therefore, to optimise the separation of the drugs from their respective products, studies to assess the effect of the mobile phase pH and acetonitrile concentration on the capacity factor of each drug were carried out. Reducing the pH of the mobile phase from 4.5 resulted in peak tailing for all four drugs. An increase in pH to 5.0 resulted in better quality peaks. Addition of silanol blocking agent (TMA) to the mobile phase further improved the sharpness of the peaks. The influence of acetonitrile concentration had a profound effect on the retention times. The change, even by 1%, led to either inadequate separation or unacceptably high elution periods. Finally, the optimum separations for the four drugs was achieved by (i) increasing the pH of mobile phase to 5.0, (ii) adjusting the ratio of the organic modifier in the mobile phase between 18 and 21% and (iii) adding 10 m*M* of tetramethylammonium chloride to the mobile phase. Table 1 gives the chromatographic conditions under which good separations were achieved at all three pH conditions for the four drugs. The retention times and capacity factors are also included.

Table 1
Chromatographic conditions for separation of four isoxazolyl penicillins from degradation products formed in acidic, neutral and basic conditions

Drug	MeCN in mobile phase (%)	Retention time (min)	Capacity factor
Cloxacillin	18.0	31.2	8.47
Dicloxacillin	21.0	31.8	8.82
Flucloxacillin	19.0	33.9	8.83
Oxacillin	15.0	35.5	9.59

Column, 300×3.9 mm Phenomenex C_{18} 5 μm; flow-rate, 1.0 $ml\,min^{-1}$; detection wavelengths, 220, 240 nm; mobile phase, MeCN: 20 m*M* potassium dihydrogen phosphate and 10 m*M* tetramethylammonium chloride in water (pH 5.0).

Typical chromatograms obtained using the optimised conditions are shown in Figs. 2 and 3. The figures are the characteristic outputs obtained when the detector is lit in a dual-wavelength mode and the data processor has the provision for two-channel recording. The recording on the lower side is the normal chromatogram output at one wavelength and printed simultaneously on its top is the ratio chromatogram of two wavelengths. Fig. 2 shows the separation of FLX from products formed under acid, neutral and basic alkaline conditions. Similar separations were observed for OLX, CLX, and DLX. The typical separation behavior of the latter three drugs in acidic conditions, where more degradation products are formed, is shown in Fig. 3. The chromatograms show clear separation of drug peaks from other peaks.

3.2. Test for homogeneity of drug peak

A test of homogeneity was subsequently applied to the separated drug peak to confirm its single component character. Among the techniques available for the purpose, which include ratio chromatography [10–12], derivative spectroscopy [13], photo diode-

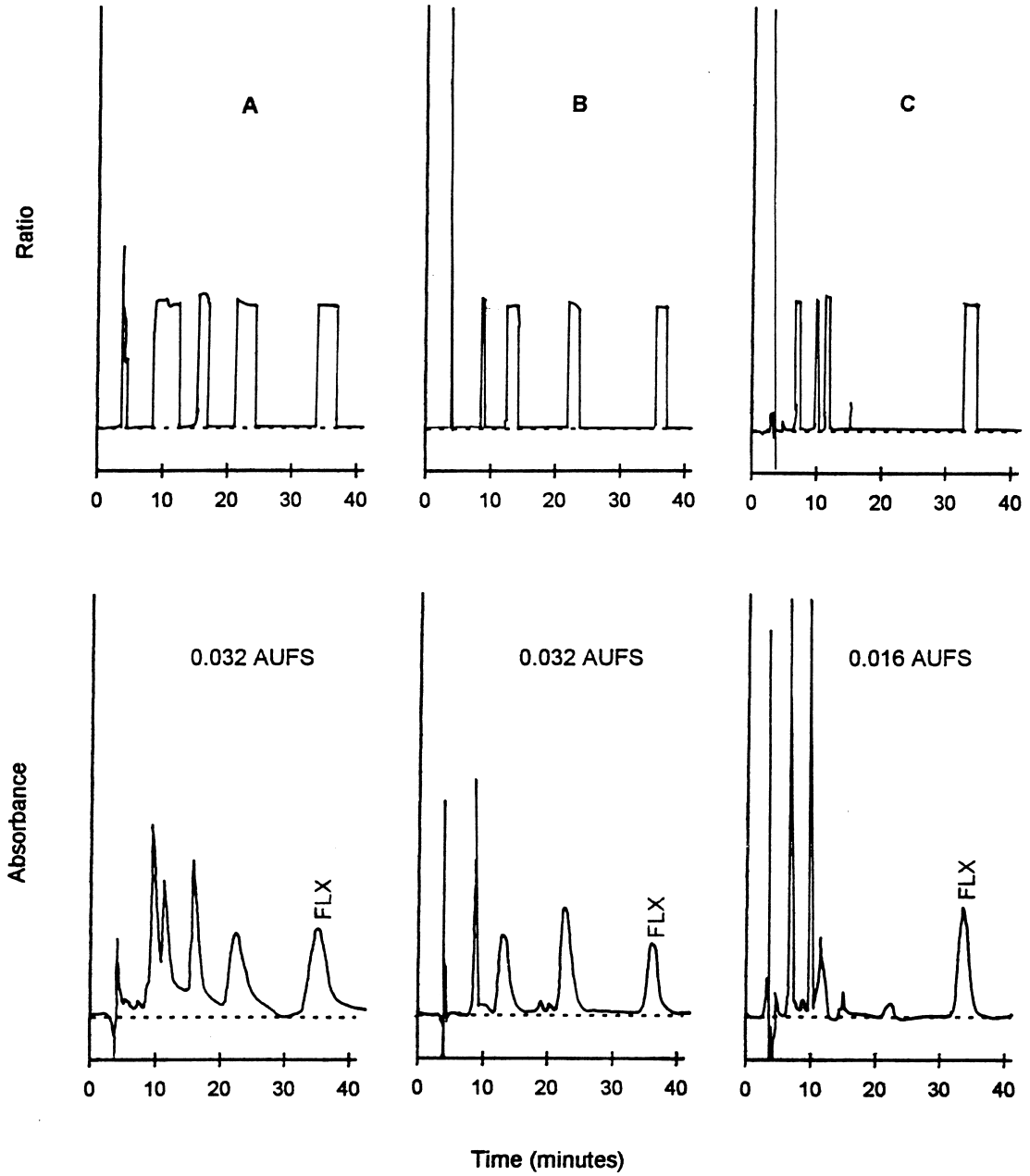

Fig. 2. Dual wavelength chromatograms obtained after approximately two half-lives during decomposition of FLX at pH (A) 2.0, (B) 7.0 and (C) 9.2. Chromatographic conditions as in Table 1.

array detection [14,15], LC–MS [15], etc., the first one was employed in our studies. The ratio plots, as obtained on the upper side in Figs. 2 and 3, accrue from the relative absorbance variations in $A_{\lambda 1(t)}$ and $A_{\lambda 2(t)}$ at the two wavelengths λ_1 and λ_2 as per the following equations [9]:

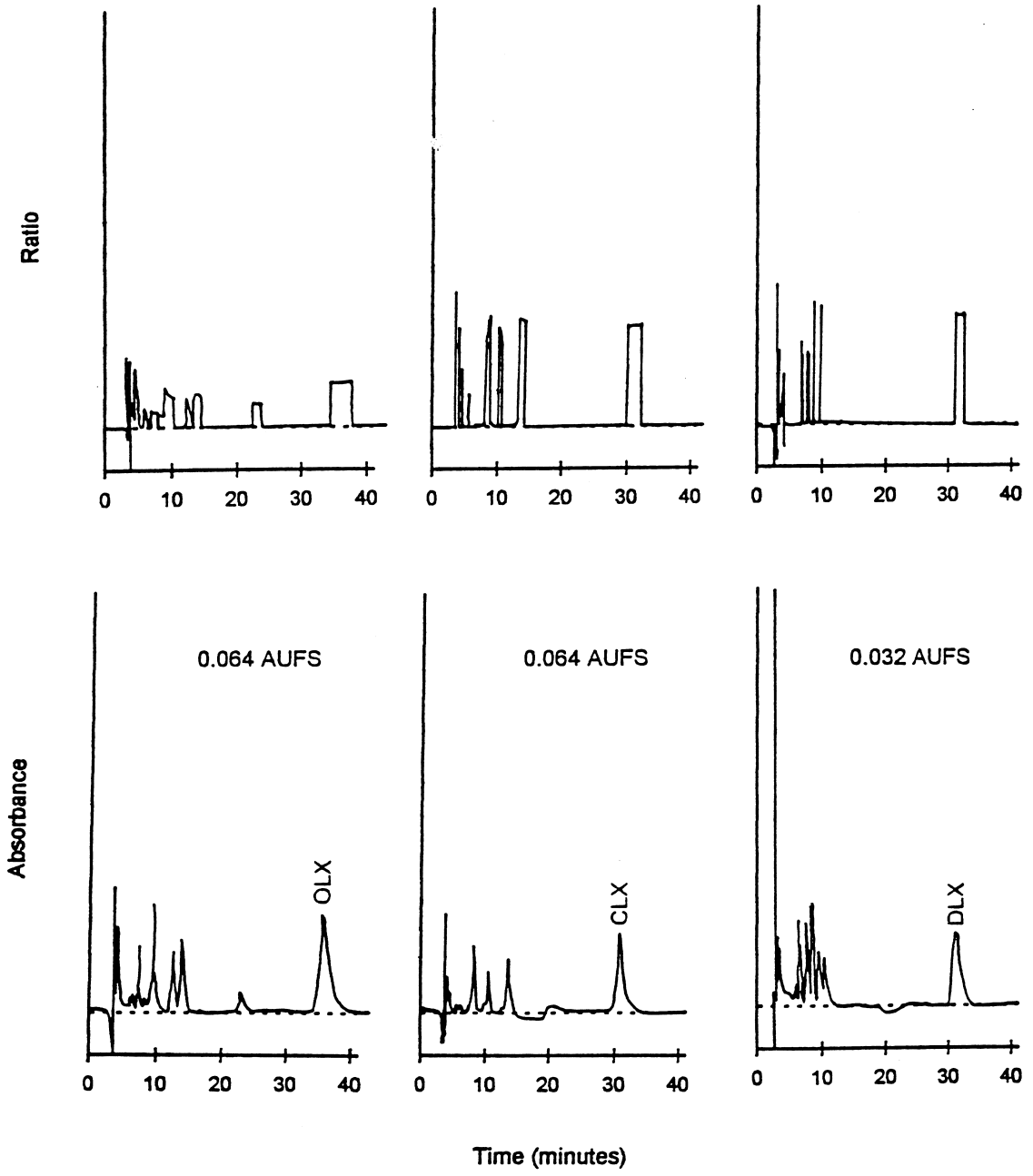

Fig. 3. Dual wavelength chromatograms obtained after approximately two half-lives during decomposition of OLX, CLX and DLX at pH 2.0. Chromatographic conditions as in Table 1.

$$R(t) = \frac{A_{\lambda 1(t)}}{A_{\lambda 2(t)}} - 1 \quad \text{when} \quad A_{\lambda 1(t)} > A_{\lambda 2(t)} \tag{1}$$

$$R(t) = 1 - \frac{A_{\lambda 1(t)}}{A_{\lambda 2(t)}} \quad \text{when} \quad A_{\lambda 1(t)} < A_{\lambda 2(t)} \tag{2}$$

where $R(t)$ is the ratio chromatogram signal. Since baseline drifts $D_{\lambda 1}$ and $D_{\lambda 2}$ may be contained at times in $A_{\lambda 1}$ and $A_{\lambda 2}$, the ratio chromatogram is actually obtained from the following equation:

$$R(t) = \frac{A_{\lambda 1(t)} + D_{\lambda 1}}{A_{\lambda 2(t)} + D_{\lambda 2}} - 1 \tag{3}$$

The ratio chromatogram for a pure compound, as per Eqs. (1) and (2), is output as a flat rectangular peak. The flatness appears because the ratio, $R(t)$, at two wavelengths remains constant. The flatness is an ideal situation and requires zero baseline drifts during elution. When the baseline drifts are included in the measured absorbances, the peak containing no impurities shows symmetrical distortions, in accordance with Eq. (3). When a peak contains an impurity, the ratio $R(t)$ varies, and the shape of the ratio chromatogram is distorted asymmetrically.

In both Figs. 2 and 3, the ratio chromatograms corresponding to drug peaks are flat in shape which indicates homogeneity of these peaks. Similar behaviour of ratio plots was observed in all studies.

The two wavelengths used in the present study for dual wavelength analysis were 220 and 240 nm. At these wavelengths all four drugs absorb strongly while the mobile phase containing acetonitrile and buffer is devoid of any absorbance. Therefore, these wavelengths were selected since they met the basic requirement for obtaining ratio chromatograms with little distortion [9].

3.3. Kinetics plots

The sensitivity of the dual-wavelength chromatographic technique to assess homogeneity throughout the peak profile depends, inter alia, on the magnitude of the difference in absorbance spectra, molar absorptivities, relative concentrations and retention times of the main compound and coeluting components/impurities [16,17]. When the difference is small, a flat ratio chromatogram may be obtained for overlapping peaks. This is considered a distinct possibility during drug degradation studies as products which are sometimes closely related in structure to the parent drug and with similar absorption characteristics are generated. Since multiple unidentified degradation products were formed during hydrolysis of isoxazolyl penicillins, a further confirmatory test of the stability-indicating nature of the method was applied in our studies. The log percent drug remaining values calculated from the peak areas obtained during the course of the decomposition kinetics studies were plotted against time. The plots were linear ($r^2 > 0.99$) for all four drugs under different pH conditions (Fig. 4). This confirmed the specificity of the methods.

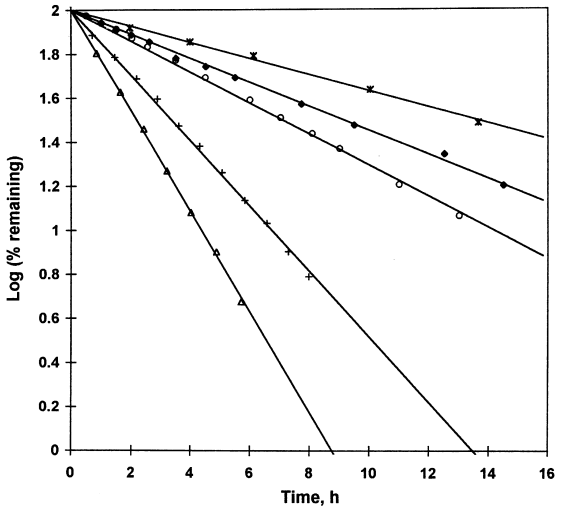

Fig. 4. Semilogarithmic plots of percentage drug remaining versus time during decomposition of isoxazolyl penicillins (○) DLX at pH 9.2, (♦) FLX at pH 9.2, (*) FLX at pH 7.0, (△) OLX at pH 2.0 and (+) CLX at pH 2.0.

4. Conclusions

Chromatographic conditions are described for specific determination of four isoxazolyl penicillins viz., CLX, DLX, OLX and FLX, in presence of their respective degradation products. The methods were developed employing a three-pronged strategy involving: (i) separation of drug from products on a chromatographic column, (ii) verification of the

purity of drug peak through a peak homogeneity test and (iii) confirmation of the stability-indicating nature of the method through kinetics studies. This approach seems satisfactory, especially in situations where the identity of decomposition products is not established and the degradation product standards are not available.

The study highlights the potential role of dual-wavelength chromatography in the development of stability-indicating assays. In view of the advantages offered, it is suggested that those who wish to buy new HPLC systems for stability test purposes, should consider spending a little extra and buy a detector with dual/multiple wavelength detection along with a compatible data processor.

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