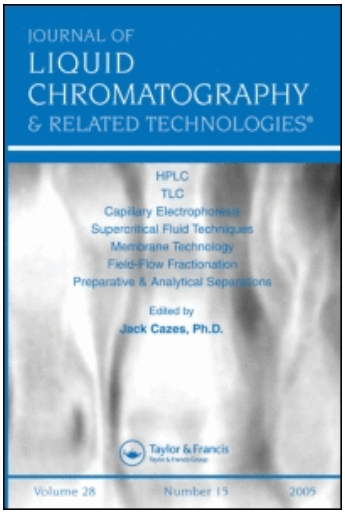

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Comparison of Techniques for Peak Purity Testing of Cephalosporins

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COMPARISON OF TECHNIQUES FOR PEAK PURITY TESTING OF CEPHALOSPORINS

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Abstract :

The efficiency of spectral suppression, absorbance ratio and spectral overlay for peak purity testing using a diode array detector has been compared on a strongly overlapping model system (cefotaxime and theophylline). Spectral overlay and spectral suppression have a comparable sensitivity (0.3 % of impurity can be detected) ; spectral suppression allows an accurate determination of impurity at 1 % level ; absorbance ratio is the less powerful technique.

INTRODUCTION

Among the criteria used for validating a HPLC method for drug analysis, specificity (i.e. the ability of the method to discriminate between the analyte and interfering substances) plays a major role. For cephalosporins, potential interfering substances may include excipients, synthesis

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intermediates and decomposition products in pharmaceutical formulations, or biological matrix, metabolites or drugs concomitantly administered, in biological fluids (See e.g. ref 1-3 and ref therein). The specificity is generally assessed by injecting a blank sample and/or suspected interfering compounds, but this procedure is limited in scope to previously identified compounds and/or the availability of the matrix. For a stability-indicating assay, the profile of a solution degraded under stress conditions of temperature, light, oxidation and pH is also examined by comparison with a reference sample. However, a critical issue for processing validation for specificity is the homogeneity of the chromatographic peaks.

The present work is a complement of previous studies[1-3] related to the development of liquid chromatographic methods for the determination of cephalosporins in pharmaceutical formulations and biological fluids. The efficiency of spectral suppression (SS) absorbance ratio (AR) and spectral overlay techniques for peak purity testing using a diode array detector has been evaluated. For this purpose, a strongly overlapping model system consisting of cefotaxime (C) and theophylline (T) has been chosen. Theophylline has been selected as a model impurity since it can be co-administered with cephalosporins in hospitals and may be encountered in the chromatograms from biological fluids. The relative proportions of the two drugs have been varied from 1 to 100 (w/w) within a concentration range of biomedical interest. Premises

of SS, AR and spectral overlay techniques have been extensively described in ref 4-6.

EXPERIMENTAL

Reagents and materials.

Cefotaxime, (Roussel UCLAF laboratories, Romainville, France) and theophylline (Cetrane-Unicet laboratories, Levallois Perret, France) were used as received. Potassium phosphate (Analar grade, BDH Chemicals, Poole, UK) methanol (HPLC "S" grade, Rathburn Chemicals, Walkburn, UK) and glass distilled water were used.

Solutions.

Stock aqueous solution of C (100.0 mgl^{-1}) and T (100.5 mgl^{-1}) were prepared. From these solutions, five solutions containing various proportions of C with respect to T and conversely, were prepared in the mobile phase below mentionned. The concentrations were respectively 0.2, 1.0, 5.0, 10.0, 20.0 mgl^{-1} of C (or T) in the presence of 20.1mgl^{-1} of T (or 20.0 mgl^{-1} of C). For linearity and recovery studies using spectral suppression (SS), pure standard solutions of each compound of the same concentration were prepared for comparison.

Mobile phase was 0.025 M phosphate buffer pH 7.0 - methanol (85 + 15) (v/v). It was filtered through a 0.45 µm Millipore filter (Bedford, MA, USA) and degassed under reduced pressure in an ultrasonic bath for 10 min.

Apparatus.

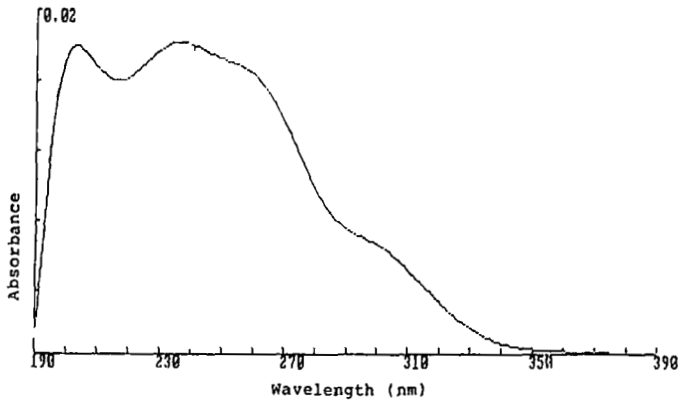
A Spectroflow 400 Kratos high performance liquid chromatograph (Ramsey, NJ, USA) provided with a 20 - µl loop was used. The chromatograph was equipped with a 10.0 x 0.8 cm Waters (Milford, MA, USA) radial-pak cartridge packed with 4 µm C_{18} Nova-pak stationary phase. The mobile phase was pumped through the column at a flow rate of 2.5 $ml.min^{-1}$. The pressure was 98 ± 1 bar. The diode array detector was a 1000 S Applied Biosystem (Ramsey, NJ, USA) with a 6.0 µl illuminated volume and a 8 mm pathlength flow cell. The spectral bandwith was set at 5 nm and the detector sensitivity ranged from 0.001 to 0.02 AUFS.

RESULTS AND DISCUSSION

Typical retention times were 3.92 and 4.04 min for cefotaxime (C) and theophylline (T) respectively, yielding a resolution factor of 0.17 for equal concentrations of C and T (20 mgl^{-1}).

Selection of the wavelengths for SS and AR.

A critical issue for SS and AR is the selection of a pair of wavelengths for selectivity and sensitivity. Since both compounds are known, the optimum pair of wavelengths could be approached from ratioing the spectra of the pure compounds in the mobile phase[5-6]. The plot of the ratio of the spectrum of C (Fig. 1a) and T (Fig. 1b) is shown in Fig. 2.



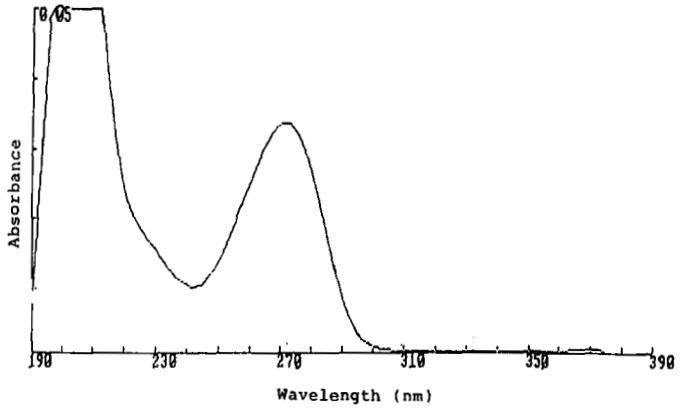


Figure 1. Absorbance spectrum by HPLC-UV detection.
a : 20.0 mgl^{-1} cefotaxime solution injected. Sensitivity, 0.02 AUFS. Other conditions, see text.
b : 20.1 mgl^{-1} theophylline solution injected. Sensitivity, 0.05 AUFS. Other conditions, see text.

A maximum and minimum absorbance ratio is observed at 240 nm and 275 nm respectively. At these wavelengths, the absorbance ratios differ most and moreover, these wavelengths are very close to the maximum absorbance wavelengths of C and

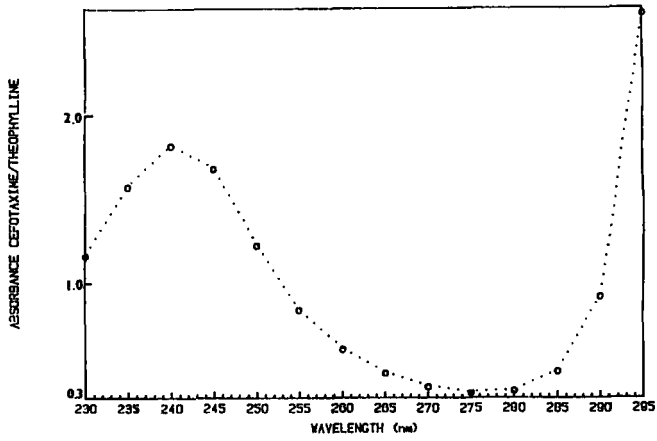


Figure 2. Graph of absorbance ratio of cefotaxime (20.0 mgl^{-1})/theophylline (20.1 mgl^{-1}) *vs* wavelength.

T, respectively. Therefore, they were selected in the further stages of the method.

Spectral suppression.

The SS constants K_C and K_T were calculated from the absorbance measurements from the injection of a solution of the pure compounds (100 mgl^{-1}). The calculated constants were $K_{T,240,275} = 0.29_1$; $K_{C,240,275} = 1.58_2$ (or $K_{C,275,240} = 0.63_2$). Using these constants, a simultaneous determination of C and T could be carried out in two different channels. If K_C is used, C is suppressed and T is monitored, and conversely.

The equation applied for suppressing C and determining T was : $\Delta A_{275,240} = A_{275} - 0.63_2 A_{240}$ (eq. 1), where ΔA is the difference absorbance at 275 and 240 nm, and A_{240} and A_{275} the absorbances measured at these two wavelengths, respectively.

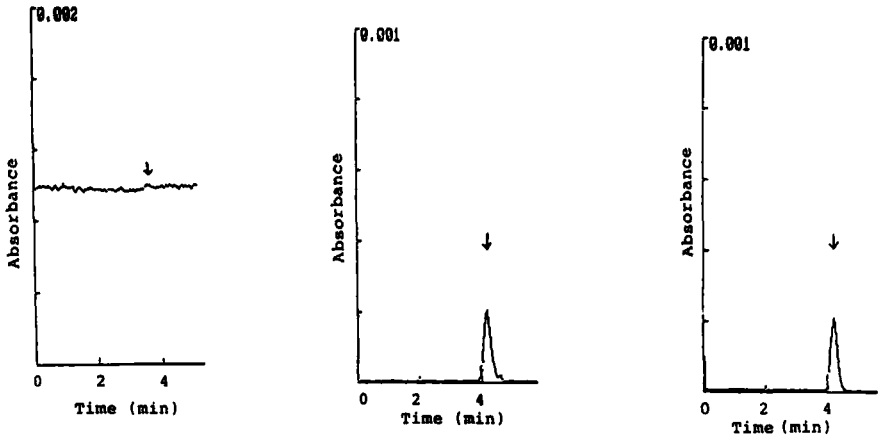


Figure 3. Spectral suppression of cefotaxime.
a : 20.0 mgl^{-1} C solution injected (offset 0.001 AU).
b : Chromatogram of T from a mixed solution of T (0.2 mgl^{-1}) and C (20.0 mgl^{-1}).
c : Chromatogram of a standard solution of T (0.2 mgl^{-1}). Equation 1 applied. Conditions, see text.

Using eq. 1 the signal of a pure solution of C (20.0 mgl^{-1}) is suppressed (Fig. 3a). Fig. 3b shows the chromatogram of T from a mixed solution of T (0.2 mgl^{-1}) and C (20.0 mgl^{-1}) after SS of C. The chromatogram of a pure solution of T (0.2 mgl^{-1}) under the same conditions is given for comparison (fig. 3c).

The equation applied for suppressing T and determining C was : $\Delta A_{240,275} = A_{240} - 0.29_1 A_{275}$ (eq. 2). Using eq. 2 the injection of a pure solution of T (20.1 mgl^{-1}) does not give any detectable signal. The signal from a mixed solution of C (0.2 mgl^{-1}) and T (20.0 mgl^{-1}) after SS of T was identical to that obtained from a pure solution of C (0.2 mgl^{-1}).

Using eq. 1, a linear response was obtained with increasing concentrations of T (0.2 - 20.1 mgl^{-1}) in constant

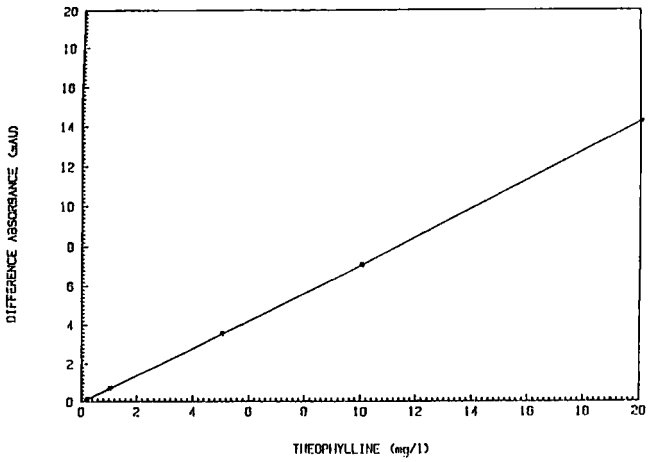


Figure 4. Calibration graph for theophylline with spectral suppression of cefotaxime (20.0 mgl^{-1}). Equation 1 applied.

concentration of C (20.0 mgl^{-1}) (Fig. 4). The regression equation was :

$\Delta A_{275,240}$ = 0.7082_2 (T) - (0.00665 • 0.02115), with a correlation coefficient of 1.000 (n = 5). Correlatively, the interference graph (Fig. 5) obtained using eq. 2 gaves a mean recovery of 99.0 % for C with a standard deviation of 0.5 % (n = 5). The limit of determination for T was lower than 1 % (w/w) with respect to C with a 400 ng loading of C on column (fig. 3b) and the limit of detection (S/N ≃ 3) was about 0.3 % (w/w).

Linearity and interference graph were also satisfactory for solutions containing increasing concentrations of cefotaxime (0.2-20 mgl^{-1}) in constant concentration of T (20.1 mgl^{-1}). The regression equation was :

$\Delta A_{240,275}$ = 0.95485 (C) + (0.02604 ± 0.08281) with a correlation coefficient of a 0.999_9 (n = 5).

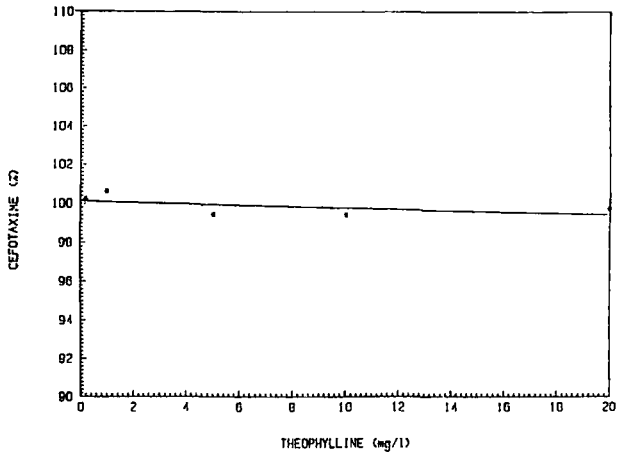


Figure 5. Interference graph showing the recovery of a constant concentration of cefotaxime (20.0 mgl^{-1}) as a function of theophylline concentration. Equation 2 applied.

The interference graph gave a mean recovery of 99.7 % for T with a standard deviation of 0.6 % (n = 5).

The limit of determination for C was about 1 % (w/w) with respect to C with a 400 ng loading of C on-column and the limit of detection (S/N = 3) was about 0.4 % (w/w).

Absorbance ratio plot.

AR was plotted for comparison. The absorbance ratio A 275/240 plotted along the elution profile allows to detect easily 5 % (w/w) of T (Fig. 6a) with respect to C but the detection of 1 % (w/w) of T is more problematic (Fig. 6b).

Spectral overlay.

The sensitivity of spectral overlay was also considered. Spectra acquired at the upslope, the apex and at the

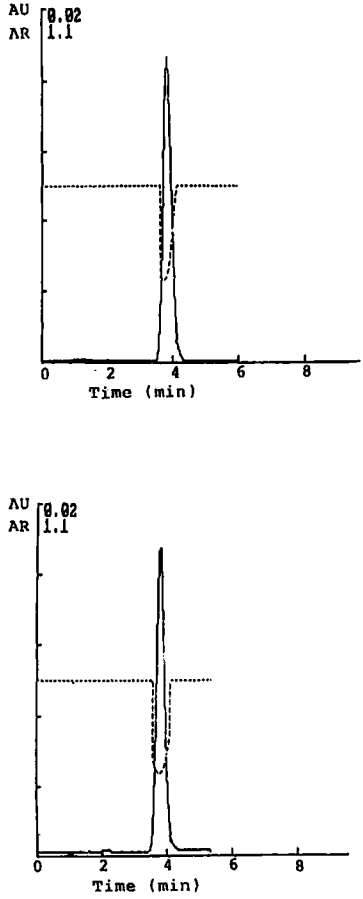


Figure 6. Absorbance ratio plots (dashed lines) from a mixed solution of cefotaxime (20.0 mgl^{-1}) and theophylline.
a : 1.0 mgl^{-1} of T.
b : 0.2 mgl^{-1} of T.
Chromatograms recorded at 260 nm (plain line). Other conditions, see text.

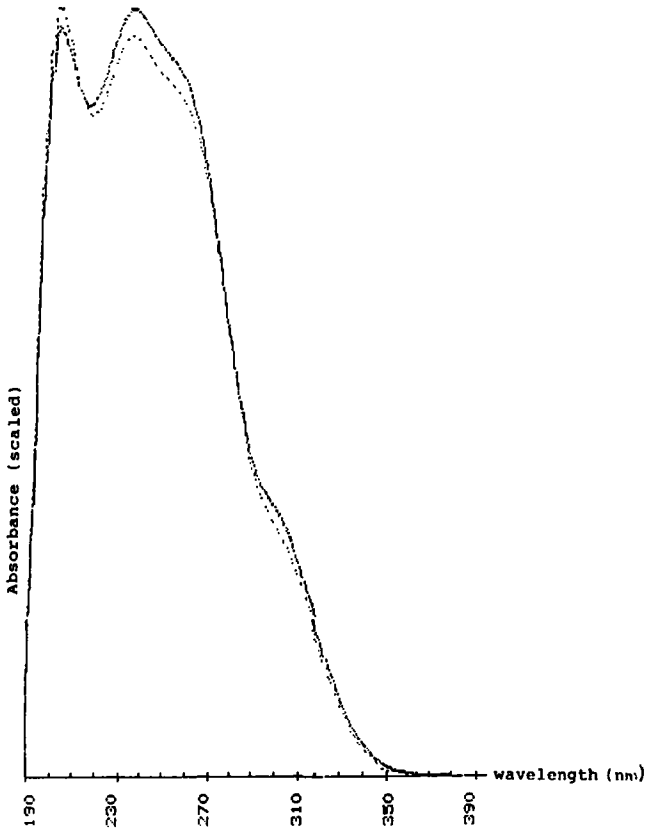


Figure 7. Spectral overlay from a mixed solution of cefotaxime (20.0 mgl^{-1}) and theophylline (0.05 mgl^{-1}).

downslope of the peak were normalized and overlayed. Fig. 7 shows that 0.25 % (w/w) of Th. in C are easily detected.

Sensitivity.

As the sensitivity of a detector plays a major role in monitoring drugs or decomposition products at trace levels,

the sensitivity of the diode array detector used in the present study was compared to that of a conventional variable wavelength detector (Kratos model Spectroflow 783, with a 12 µl volume, and a 8 mm pathlength flow cell). The study was carried out on C and four of its potential decomposition products[1]. The compounds were separated on the same C_{18} stationary phase with a 0.025 M phosphate buffer pH 7 - methanol 89 + 11 (v/v) mobile phase at a flow rate of 1.5 ml/min.

Under the same chromatographic conditions, the limit of detection was about two times lower using a conventional detector relative to the diode array detector. This shows that spectral information on contemporary diode array detector is not gathered at the expense of detector sensitivity.

CONCLUSION

The present study is an essential prerequisite for the determination of cephalosporins in complex matrices. It has shown that SS is a powerful technique for testing peak purity both qualitatively and quantitatively. SS presents several advantages by comparison to AR plot. i/ the interpretation is easier because an excursion in the baseline is easier to monitor than a shift in a square wave ii/ it can be applied to completely co-eluted compounds iii/ if the spectral characteristics of both the analyte and the impurity are known a

simultaneous determination of the two compounds can be carried out with accuracy. The sensitivity of the method depends both on the chromatographic resolution and the magnitude of difference in the absorptivities of the compounds at the selected wavelengths. When it is included in the software of a diode array detector, SS can be easily applied on a routine basis.

Spectral overlay technique has a sensitivity comparable to SS, does not require information on the spectra but cannot be used quantitatively.

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