DOXORUBICIN PLASMA DETERMINATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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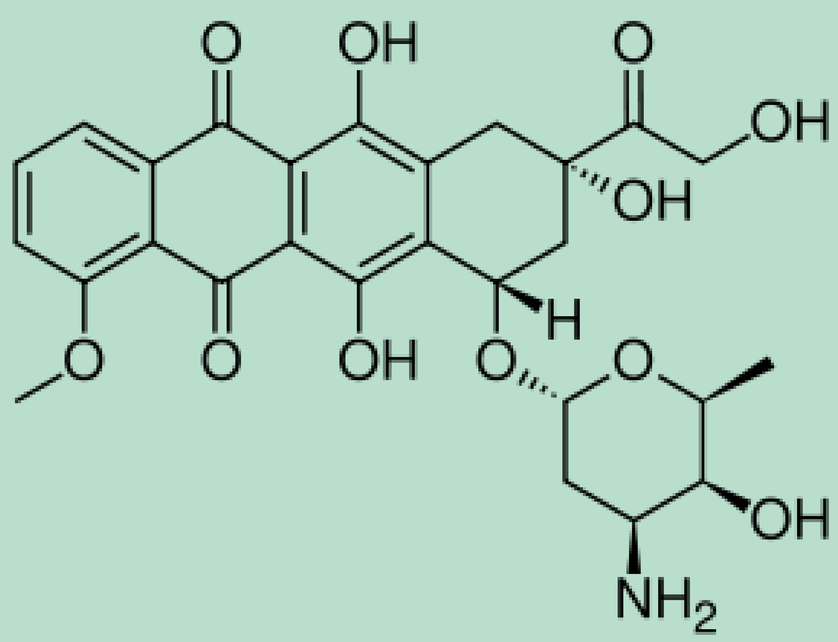
PURPOSE

✧ To assess and validate the chromatographic conditions for determining plasma doxorubicin (DXR). Linearity, accuracy, precision and reproducibility inter- and intra- assay were studied.



MATERIAL AND METHODS

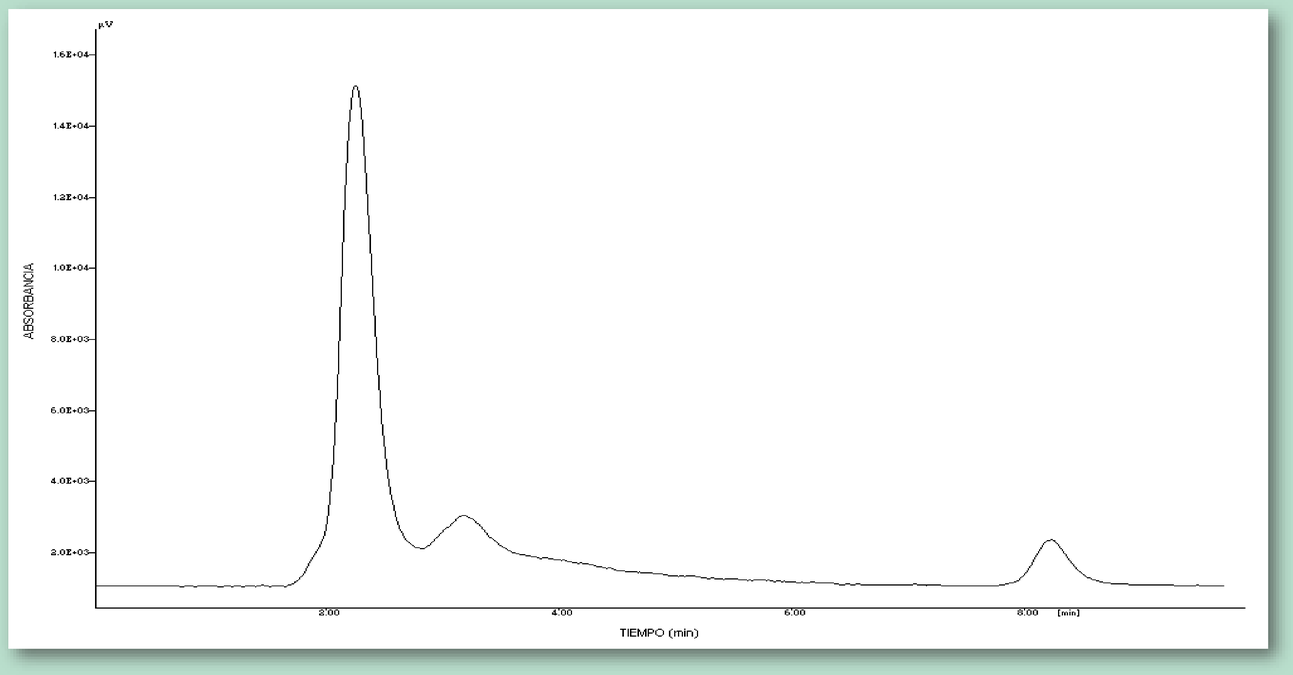
The test products used were daunorubicin (internal standard) and DXR (standard substance). The reagents used were potassium dihydrogen phosphate, acetonitrile, water and isopropanol. Free human plasma drug was provided by the hospital laboratory analysis.

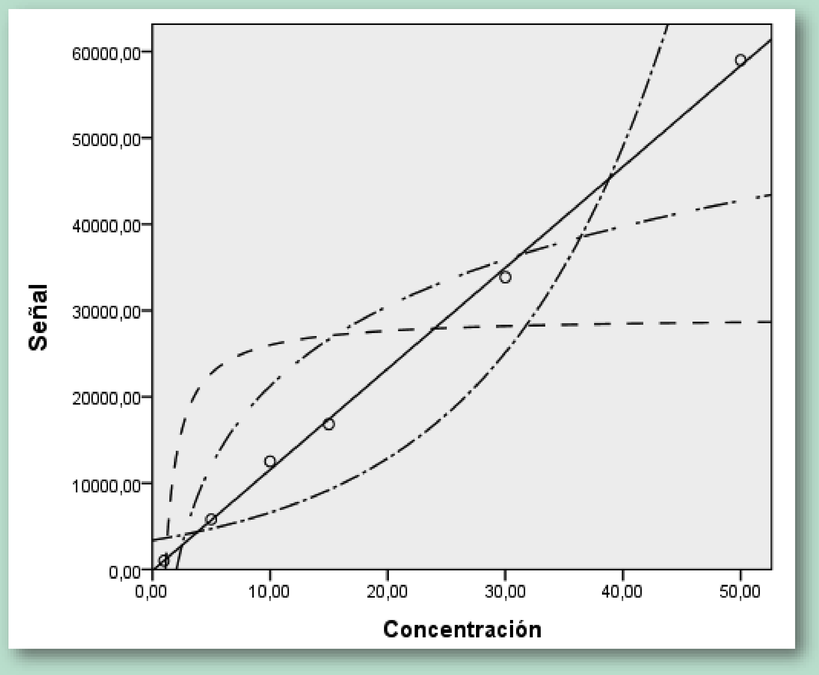
Equipment used in the study: a modular system of high performance liquid chromatography (HPLC) Merck-Hitachi composed of a pump, autoinjector system, fluorescence detector, integrating software and a computer. A centrifuge and a vortex were also used.

The stationary phase used was a 5µm C18 chromatographic column 150 mm × 4 mm, and the selected mobile phase was 0.05 M potassium dihydrogen phosphate (pH=3.55) and acetonitrile 70:30 (v/v). The flow rate chosen was 0.6 mL/min and the wavelengths of excitation and emission were 548 nm and 470 nm.

RESULTS

The equation of the calibration curve (peak area and plasma DXR) was: y=-256,34 + 1231,27 x. The analytical technique had good linearity. With 95% confidence it can be said that the intercept was between 162.4 and 350.3 area/C. With a probability of 99.5% the value obtained and the actual value were not statistically different, therefore the method has the necessary accuracy. The requirements of precision (repeatability and reproducibility) were also met. The coefficients of variation of plasma concentrations did not exceed 10% for either intra or inter studies (repeatability and reproducibility).





CONCLUSION

The chromatographic technique developed to determine plasma DXR is a quick and simple technique that meets all of the requirements of specificity, linearity, accuracy and precision required for validation.