Internal standard. Should the analytical method utilize an internal standard, it is not recommended to degrade the internal standard, but its k' should not interfere (elute) at any of the possible eluting k' peaks.

Evaluation of the degradation mixture is generally performed using a photodiode array detector. Assessing the purity of the major peak is very important and could be difficult in light of possible peak inhomogeneity after the degradation process. One must be assured that there is no degradation peak (hiding) under or unresolved from the major peak of interest. The utility of the diode-array detector is that the analyst can select a whole wavelength range, say from 200 to 350 mm, with a bandwidth of 80 nm. With just one single chromatographic run, all compounds absorbing within this range will be detected. With only one wavelength selected using a conventional UV detector, for instance at 280 nm, any compound not absorbing at this wavelength will not be detected. Figures 1A and 1B depict diode-array chromatograms for assessing peak purity.

Refer to Section 17.4 for further discussion.

8. PEAK PURITY

There is always that nagging question of whether the peak of interest (the major analyte peak) is pure or homogeneous. This is a difficult question, and many investigators have tried to prove the homogeneity of the major peak under stressed conditions during the method development and validation process. Various techniques have been used to characterize peak homogeneity, such as spectral suppression, absorbance ratio, spectral overlay (13), electrospray mass spectrometry (14,15) and dual detection (16).

9. SAMPLE PREPARATION

Sample preparation is a critical step in the overall chromatographic process, and can affect the chromatography if not developed or treated properly. This step encompasses sample filtration, sample extraction as well as sample derivatization, although the latter is not commonly used in the pharmaceutical quality laboratory. The purpose of this step is to prepare the sample so that the drug substance can be readily chromatographed, separated from other materials. Thus, it is a step to remove any interferences, to enhance the detection of the drug substance as well as to protect or enhance the life of the analytical column.

The following considerations are noted:

What is the matrix?
Ensure complete dissolution of the analyte in mobile phase or weaker solvent.
Miscibility and solubility.
Does the analyte precipitate in the buffer?
Some typical treatment modes are
- Direct injection
- Dilution
- Sonication
- Shaking