On rare occasions, the drug product, in a solution package form, may be injected directly or after an appropriate dilution. Typically, for a solid, such as a tablet or a capsule, the pretreatment would necessitate a comminution step, followed by extraction/sonication, filtration, and dilution. For example, an ointment may necessitate an extraction followed by evaporation, reconstitution, and dilution, or heating to dissolve the analyte/matrix, followed by cooling to precipitate the matrix, and then filtration.

While the typical dosage form—solid (tablet/capsule), semisolid (ointment/cream), or solution (cough syrup/ophthalmic solution)—utilizes a combination of the treatment modes mentioned earlier, solid-phase extraction (SPE) has become a recognized and viable technique for sample preparation methodologies, especially for biosamples and as an alternative to liquid-liquid extractions in many U.S. Environmental Protection Agency (EPA) methods. A recent supplement to *LC/GC* magazine was dedicated to advances in SPE (17).

It is very important that the sample preparation, prior to injection into the liquid chromatograph, be freed of particulate matter, through either filtration or centrifugation, and that the solvent be compatible with the HPLC system. If there is incomplete sample solubility or if the solvent is too polar, band distortions or tailing will result. Ensuring that the sample is completely dissolved in the proper solvent and then diluting the sample in mobile phase will eliminate these problems.

10. DEVELOPING THE SEPARATION—CHOOSING THE EXPERIMENTAL CONDITIONS

From Sec. 5, we assume that separation goals have been determined, such as resolution (at least baseline), reasonable run time (under 10 minutes), and ruggedness. These elements are further discussed below and developed in greater depth in Part II of this chapter under Validation. From Sec. 6.2 above, a case has been made that reversed-phase HPLC is suitable for our API of interest. The next step is to determine whether the API is typical. Referring to Secs. 6.1 and 6.2 above, let us further assume that the API is ionic and acidic. From a listing of generic separation conditions, see Table 3, conditions for an ionic and acidic compound are selected, and an initial exploratory run using gradient elution is made.

At this point, two options may be available to us before performing the exploratory run in the development of the desired stability-indicating procedure. First, there may be a method, either in-house or from the literature, already available for the same API or compound of interest. Useful information may be gleaned from here to modify to suit the specific compound on hand. On the other hand, sometimes established methods may not be optimal, so rather than modifying the method to suit our need, it may be better in the long run to develop a new method that is optimal and rugged.

Exploratory runs can be done manually or with computer software. Both are trial and error methods, but the latter is more systematic, quicker, and requiring fewer injections. When and after an initial exploratory run has been performed, the chromatogram is evaluated before proceeding with the next injection, and subsequent adjustments are made to the mobile phase composition. Each subsequent injection is thus based on the previous conditions, so that after a number of injections