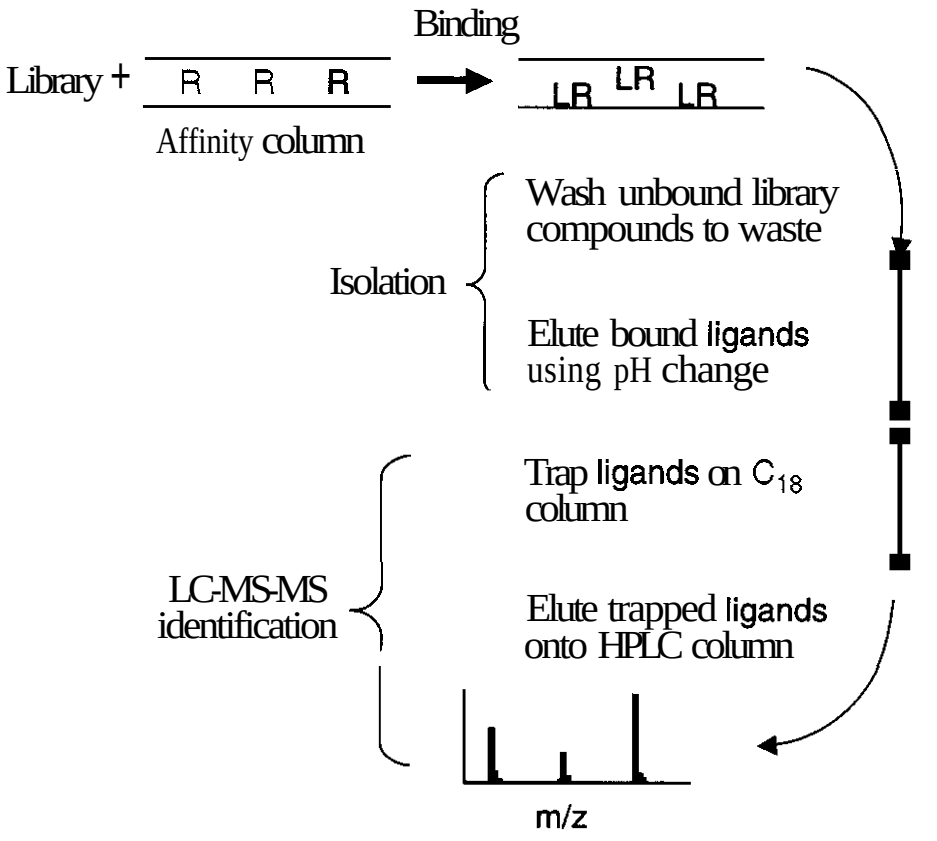

Figure 13.7. Affinity chromatography combined with **LC-MS-MS** for screening combinatorial library mixtures.

source of molecular diversity for drug discovery. All of the mass spectrometry–based screening methods use receptor binding of ligands as the basis for identification of lead compounds.

2.4.1 Affinity Chromatography–Mass Spectrometry. Since the introduction of affinity chromatography more than 30 years ago, this technique has become a standard biochemical tool for the isolation and identification of new binding partners to specific target molecules. Therefore, the coupling of affinity chromatography to mass spectrometry is a logical extension of this technique, and the application of affinity LC-MS to the screening of combinatorial libraries has been demonstrated by several groups (43, 44). During affinity LC-MS screening, a receptor molecule such as a binding protein or enzyme is immobilized on a solid support within a chromatography column. The library mixture is pumped through the affinity column in a suitable binding buffer so that any ligands in the mixture with affinity for the receptor would be able to bind. Then, unbound material is washed away. Finally, the specifically bound ligands are eluted using a destabilizing mobile phase and identified using mass spectrometry. This affinity-column LC-MS assay is summarized in Fig. 13.7.

In some applications (43), ligands are eluted from the affinity column and then trapped on a second column such as a reverse phase HPLC column. LC-MS or LC-MS-MS identification of the ligands (hits) is then carried out using the trapping column. In other systems, ligands are identified directly from the affinity column using mass spectrometry (44). For example, Kelly et al. (44) prepared an affinity column containing immobilized phosphatidylinositol-3-kinase and used it for direct LC-MS screening of a 361-component peptide library. Electrospray mass spectrometry and tandem mass spectrometry were used to identify the ligands released from the affinity column using pH gradient elution.

Advantages of affinity chromatography–mass spectrometry for screening during drug discovery include versatility and re-use of the column. Both combinatorial libraries and natural product extracts can be screened using this approach, and a wide range of binding buffers may be used. Mass spectrometry–compatible mobile phases are only required during the final LC-MS detection step. Furthermore, a single column may be used multiple times to screen different samples for ligands unless the destabilization solution irreversibly denatures, releases, or inhibits the receptor.

Despite these advantages, affinity chromatography has numerous drawbacks that have