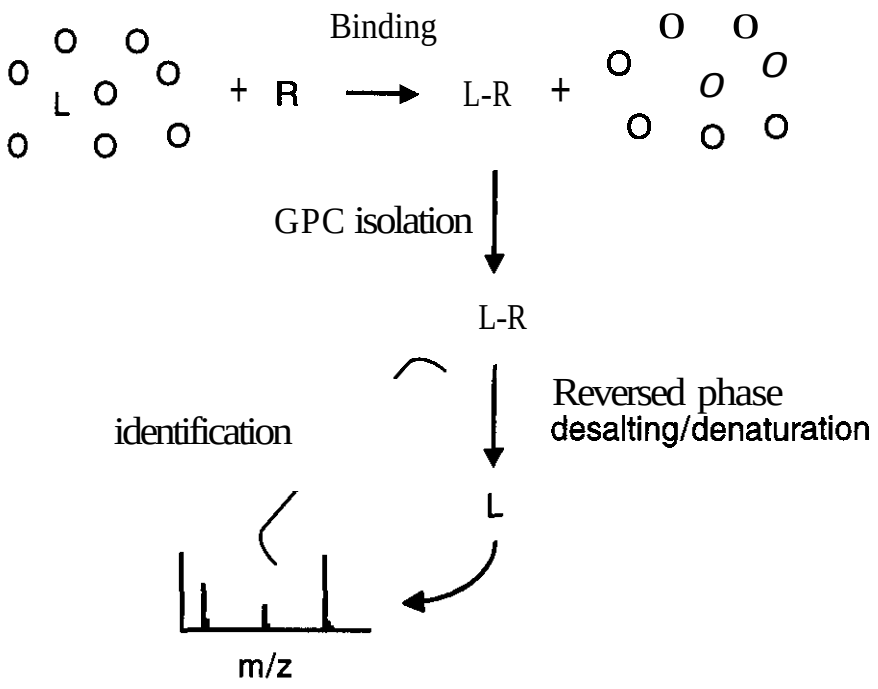

Figure 13.8. GPC followed by LC-MS-MS for screening mixtures of combinatorial libraries. After incubation of a receptor with a library of compounds, the ligand-receptor complexes (L-R) are separated from the low molecular weight unbound library compounds using GPC. Next, the L-R complexes are denatured during reversed phase HPLC to release the ligands for MS-MS identification.

prompted the development of alternative mass spectrometer screening tools. For example, immobilization of the receptor might change its affinity characteristics causing false negative or false positive hits. This is particularly problematic for receptors that are solution-phase in their native state. Also, developing and then implementing an immobilization scheme is often a slow, tedious, and even expensive process, and this process is unique for each new receptor. Finally, false positive hits are often obtained when screening large molecularly diverse libraries, because there are usually compounds in such mixtures that have affinity for the stationary phase or linker molecule instead of the receptor.

2.4.2 Gel Permeation Chromatography–Mass Spectrometry. Another type of chromatography that has been combined with mass spectrometry as a screening system for drug discovery is gel permeation chromatography (GPC) (45, 46). Also called size-exclusion chromatography, GPC separates molecules according to size as they pass through a stationary phase containing particles with a defined pore size. During GPC-based screening, a library mixture is pre-incubated with a macromolecular receptor to allow any ligands in the library to bind, and then GPC is used to separate the large receptor–ligand complexes from the unbound low molecular weight compounds in the mixture. Finally, ligands are released from the receptor during reversed phase HPLC and identified either on-line or off-line using tandem mass spectrometry. This screening method is illustrated in Fig. 13.8.

During the pre-incubation and GPC steps, any binding buffer may be used, because the binding buffer will be removed during reverse phase LC-MS analysis. However, the GPC separation step must be carried out quickly, because ligands begin to dissociate from the receptor immediately and can become lost into the size exclusion gel. Despite this disadvantage, this approach allows both receptor and ligand to be screened in solution, which avoids some of the problems associated with the use of affinity columns for screening. The GPC LC-MS-MS screening method should also be suitable for screening natural product extracts as well as combinatorial library mixtures.

2.4.3 Affinity Capillary Electrophoresis–Mass Spectrometry. Affinity capillary electrophoresis was originally used for the determination of the binding constants of small molecules to proteins (47–49). This solution-based technique is rapid and requires only small amounts of ligands. Affinity constants are measured based on the mobility change of the ligand on interaction with the receptor present in the electrophoretic buffer (50). By combining affinity capillary electrophoresis with on-line mass spectrometric detection and