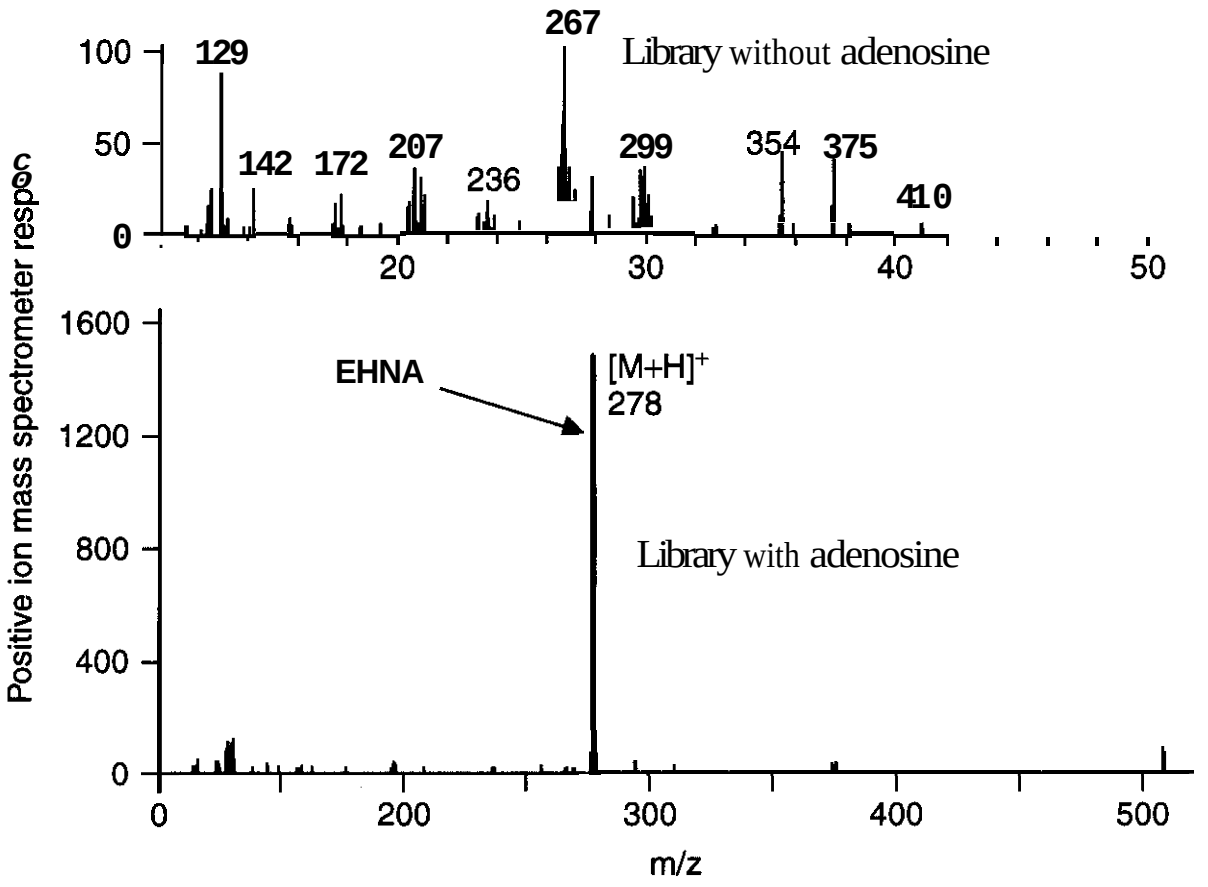

Figure 13.13. Identification of **EHNA** as the highest affinity ligand for adenosine deaminase in a combinatorial library of **20** adenosine analogs using **ultrafiltration** electrospray mass spectrometry. (Reproduced from Ref. 61 by permission of the American Chemical Society.)

combination of both. The released ligands are identified on-line using APCI or **electrospray** mass spectrometry (61) or collected and analyzed off-line using mass spectrometry, **LC-MS**, or LC-MS-MS (63).

An example of pulsed ultrafiltration mass spectrometry for the screening of a library of 20 adenosine analogs for ligands to adenosine deaminase is shown in Fig. 13.13. After a **15-min preincubation** of the library compounds (17.5 μM each except for EHNA, which was present at 1.75 μM) **with** 2.1 μM adenosine deaminase in 50 mM phosphate buffer, an aliquot **containing** 420 pmol of the receptor was injected into the ultrafiltration and washed for 8 min at 50 **μL/min** with water to remove the phosphate buffer and unbound or weakly binding library compounds. Methanol was introduced into the mobile phase to dissociate the enzyme–ligand complex and release bound ligands for identification by **electrospray** mass spectrometry. During methanol elution, only EHNA [erythro-9-(2-hydroxy-3-nonyl) adenine] was detected as the $[M+H]^+$ ion of m/z 278 (Fig. 13.13). In control experiments using the library without enzyme, no library compounds were detected during methanol elution (Fig. 13.13, Control). Despite being present at a 10-fold lower concentration than the natural substrate adenosine analogs, EHNA was easily identified because it had the highest affinity among the library compounds (K_d = 1.9 nM). This demonstrates the use of **ultrafiltration electrospray** mass spectrometry for identifying a high affinity ligand among a set of analogs that bind to a specific receptor. In a follow-up lead optimization study using pulsed ultrafiltration mass spectrometry, a **synthetic combinatorial** library of EHNA analogs was screened for binding to adenosine deaminase, and **structure-activity** relationships for EHNA binding were identified (65).

As an illustration of the versatility of pulsed ultrafiltration–mass spectrometry, binding assays for a variety of receptors have been reported including **dihydrofolate reductase (63)**, cyclooxygenase-2 (**62**), serum albumin (66, 67) and estrogen receptors (**68**). Not only is pulsed ultrafiltration useful for **identifying ligands** to different receptors, but a wide range of **combinatorial** libraries and natural product extracts in any suitable binding buffer may be screened. In addition to **combinatorial** libraries, complex natural product extracts