

## AL16

### **Development of Highly Sensitive and Selective Analytical Procedures for Steroids Causing Age-related Diseases Using LC/MS Combined with Derivatization**

**Tatsuya Higashi**

(Div. Pharm. Sci., Grad. Sch. Natl. Sci. Technol., Kanazawa Univ.)

With the rapid shift to an aging society in Japan, age-related diseases, such as osteoporosis, dementia and cancer, are increasing sharply. The measurement of steroids related to these diseases in biological fluids and tissues is useful for elucidation of the nature, diagnosis and treatment of the diseases. LC/MS employing ESI or APCI is considered to be the most promising method for this purpose due to its specificity and versatility, but it sometimes does not demonstrate the required sensitivity for the trace amount of steroids, because steroids have a rather low response using either ESI or APCI. LC/MS has another shortcoming; structural information is minimally obtained even if the MS/MS mode is used, because a conventional instrument employs soft ionization and low-energy collision. To overcome these problems, the author developed detection- and structural elucidation-oriented derivatization procedures for steroids in LC/MS; a summary of the results is shown below.

For ESI-MS, introduction of a permanently charged moiety is effective. Based on this, 2-hydrazino-1-methylpyridine was developed and used in monitoring prostatic dihydrotestosterone, a good index for the follow-up of patients affected by prostate cancer under anti-androgen therapy. Electron-capture APCI-MS based on derivatization with 2-nitro-4-trifluoromethylphenylhydrazine was applied to the analysis of neurosteroids, which affect brain excitability through action at neurotransmitter receptors. With this method, the stress-induced rapid biosynthesis of pregnane-type neurosteroids in rat brains was demonstrated. Glucuronidated conjugates of vitamin D metabolites were converted to their adducts with 4-phenyl-1,2,4-triazoline-3,5-dione, which gave a characteristic product ion indicating the conjugation position by positive ESI-MS/MS/MS, which was not observed in the intact forms.