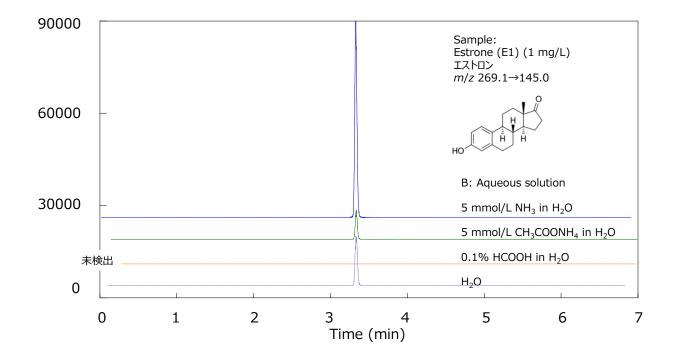
Application No. L3049

Estrogen

"In our application (L3047), we successfully improved the detection sensitivity of steroids by utilizing FIA-MS measurement with ammonia eluent. In this study, we focused on analyzing three estrogens - estrone (E1), 17- β -estradiol (E2), and ethinyl estradiol (17 α -Ethinylestradiol, EE2) - via LC-MS/MS.

During the analysis, we utilized four different eluents: ammonia, ammonium acetate, formic acid, and ultrapure water. We then compared the detection sensitivity of each eluent. Ultimately, the ammonia eluent proved to be the most effective in enhancing the detection sensitivity of the target analytes."

Key words	:	Alkaline eluent Ammonia
Column	:	USP category: L1
[Analytical conditions]		
Column	:	<i>L-column</i> 3 <i>C18</i> (3 μm, 12 nm); 2.1 mm I.D. × 50 mm L.; Cat. No. 811140
Eluent	:	A: CH ₃ CN; B: Aqueous solution
		A/B, 5/95 (0-0.5 min)-95/5 (5-7 min)
Flow rate	:	0.2 mL/min
Temperature	:	40℃
Detection	:	ESI-MS/MS(-)
Injection volume	e:	2 μL
System	:	LC: Ultimate 3000 Bio RS (Thermo Fisher Scientific K.K.); MS/MS: 3200 QTRAP (SCIEX)



After comparing chromatograms obtained from each eluent, we were able to detect each component with high sensitivity using ammonia eluent. Estradiol, in particular, is known for its low ionization efficiency. However, the use of ammonia eluent (pH 10) helped to dissociate the terminal OH group of estradiol - which has a pKa of 10.7 - and promote ionization with negative ions. The high pH of the ammonia eluent facilitated the deprotonation of the estradiol molecule, allowing for enhanced ionization. As a result, we were able to detect estradiol - and other target analytes - with greater sensitivity using ammonia eluent in our LC-MS/MS analysis.





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