K7 Mass Spectrometric Data Characteristics of 7-Aminoflunitraze-pam and 7-Aminoclonazepam With Multiple Derivatization Groups

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After attending this presentation, attendees will have an enhanced appreciation of the significance and important factors associated with the selection of derivatization reagent, internal standard, and ion pairs for GCMS analysis of drugs in biological specimen.

This presentation will impact the forensic community and/or humanity by advancing the practice in the quantification of drugs/metabolites in biological specimens.

Detecting low-levels of flunitrazepam metabolites in blood and bloodstains was reportedly facilitated by sequential derivatization with pentafluoropropionyl (PFP) and *t*-butyldimethylsilyl (TBDMS) groups [1]. Based on these findings, this study was carried out to compare the effectiveness of several groups when used in sequential derivatization of 7-aminoflunitrazepam and 7-aminoclonazepam, two benzodiazepines with more than one active site. Commercially available deuterated analogs of these two compounds, d_3-7-aminoflunitrazepam, d_7-7-aminoflunitrazepam, d_4-7aminoclonazepam, were also included in this study to determine their effectiveness as internal standards for quantification.

Trifluoroacetyl (TFA), PFP, and heptafluorobutyryl (HFB) were adapted as the first, while trimethylsilyl (TMS) and TBDMS were used as the second derivatization groups. Products resulting from the first step and the two-step derivatization processes were analyzed by GC-MS. Full-scan mass spectrometric data were used to select ions with the potential for designating the analytes and their respective deuterated analogs in quantitative analysis protocols. Selected ion monitoring data of these ions were then collected and assessed to determine whether the quality of these ions were significantly different when one or two different derivatization groups were adapted in these sample preparation processes (Table 1). A total of 54 fullscan mass spectra and 3 ion intensity cross-contribution tables, representing various forms of derivatization and isotopic analogs of these two compounds, are systematically presented for reference. Evaluations of these data concluded: (a) for 7-aminoflunitrazepam, combination of PFP/TMS derivatization with d_7-7-aminoflunitrazepam serving as the internal standard generated the most favorable ion pairs for quantification and as supporting parameters for qualitative analysis purposes; (b) data resulting from the 7-aminoclonazepam study were not as clear; however, the combination of TFA/TMS appeared to be the best choice.

Reference:

1. A.A. Elian. Detection of low levels of flunitrazepam and its metabolites in blood and bloodstain. Forensic Sci. Int. 101 (1999) 107–111.

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Table 1. Double derivatization groups, most favorable ions (*m/z*) for designating the analytes and their deuterated internal standards, and percent cross-contribution by the internal standard to the intensity of ions designated for the analyte and vise versa.

	Ions (and % cross-contritbution) designating analyte and internal standard	
Derivatization Group[a]	$d_0$and d_7-7-aminoflunitrazepam	$d_0$and d_4-7-aminoclonazepam
Ethyl/ethyl	—[b]	312 (1.70), 341 (4.78), 342 (1.41)
	—	316 (6.32), 345 (6.87), 346 (2.29)
Propyl/propyl	—	340 (0.88), 369 (0.31), 370 (0.75)
	—	344 (2.71), 373 (1.00), 374 (0.34)
Butyl/butyl	—	354 (0.38), 397 (0.23), 398 (0.64)
	—	358 (0.90), 401 (1.18), 402 (0.35)
TMS/TMS	—	394 (0.36), 414 (0.23), 429 (0.33)
	—	398 (2.53), 418 (6.00), 433 (6.47)
t-Butyl-TMS/*t*-butyl-TMS	—	456 (0.38), 457 (0.61), 458 (1.01)
	—	460 (6.59), 461 (3.84), 462 (0.77)
TFA/TMS	423 (0.35), 450 (0.43), 451 (0.44)	—
	430 (0.08), 456 (0.50), 458 (0.00)	—
TFA/*t*-butyl-TMS	436 (0.16), 437 (0.20), 493 (0.15)	—
	443 (0.01), 444 (0.28), 500 (0.00)	—
TFA/2*t*-butyl-TMS	—	552 (1.52), 553 (1.61), 554 (2.00)
	—	556 (4.11), 557 (2.33), 558 (0.42)
PFP/TMS	473 (0.17), 500 (0.24), 501 (0.17)	—
	480 (0.64), 506 (0.05), 508 (0.00)	—
PFP/*t*-butyl-TMS	337 (6.58), 486 (5.45), 543 (5.72)	—
	340 (2.49), 493 (0.02), 550 (0.00)	—
PFP/2*t*-butyl-TMS	—	602 (0.13), 603 (0.24), 604 (0.51)
	—	606 (6.71), 607 (4.75), 608 (0.85)
HFB/TMS	523 (0.37), 550 (0.27), 551 (0.27)	—
	530 (6.10), 556 (0.09), 558 (0.00)	—
HFB/*t*-butyl-TMS	296 (4.81), 536 (3.00), 537 (3.10)	—
	299 (1.92), 543 (0.22), 544 (0.43)	—
HFB/2*t*-butyl-TMS	—	652 (0.27), 653 (0.32), 654 (0.67)
	—	656 (4.97), 657 (2.57), 658 (0.63)

[a] TMS: trimethylsilyl; *t*-butyl-TMS: *t*-butyldimethylsilyl; TFA: trifluoroacetyl; PFP: pentafluoropropionyl: FFB: heptafluorobutyryl. [b] Attempts to attach the second derivatization group were unsuccessful.

Flunitrazepam, Clonazepam, GC-MS

