Development of a GC-MS method for determination of Benzodiazepine Series Drugs

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NOVELTY

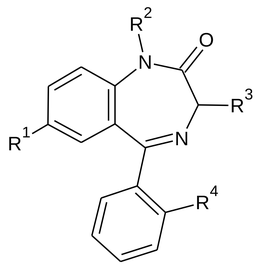
A GC-MS method for determination of drugs of benzodiazepine series is developed. Thermal and EI induced decompositions of these compounds are studied by varying sample inlet configurations and ion source systems. Reliable GC retention index (GCRI) values and mass spectral data are acquired.

INTRODUCTION

Benzodiazepines are widely prescribed drugs. They are sensitive to elevated temperature. Detection of benzodiazepines by various mass spectrometry methods, including GC-MS, involves preliminary chemical modification of these compounds yielding stable derivatives; often the spectra of derivatives exhibit fragmentation pathways useful for structure determination. In this study synthesis, measurements and examination of GCRI and EI mass spectra of trimethylsilyl derivatives of these drugs of abuse are conducted. Acquired data will be included in the 2017 release of the NIST/EPA/NIH library.

EXPERIMENTAL

Chemicals: Lorazepam (***1***), Lormetazepam (***2***), N-Desmethylflunitrazepam (***3***), Clonazepam (***4***), Oxazepam (***5***), Temazepam (***6***) are commercially available.



(***1***) R^1,R^4= Cl; R^2= H; R^3= OH. (***2***) R^1,R^4= Cl; R^2= CH_3; R^3= OH.
(***3***) R^1= NO_2; R^2,R^3= H; R^4= F. (***4***) R^1= NO_2; R^2,R^3= H; R^4= Cl.
(***5***) R^1= Cl; R^2= H; R^3= OH; R^4= H.
(***6***) R^1= Cl; R^2= CH_3; R^3= OH; R^4= H.

Trimethylsilylation was carried out at 60°C with the use of N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) or N-Methyl-N-(trime thylsilyl)trifluoroacetamide (MSTFA) as silylating reagents. On-column derivatization of Lorazepam and Oxazepam was performed to identify any thermal decomposition products (Figure 1).

Instrumentation and measurements

Standard GC-MS method: EI mass spectra were measured on GC-MS systems with quadrupole analyzers (ionization energy 70 eV; ion source temperature 230ºC), with GC conditions: VF-5ms capillary column (30 m, 0.25 mm, 0.25 µm, 1 mL/min helium (He) flow); oven temperature program – from 60°C to 270°C at 10°C/min; inlet and transfer line at 270°C.

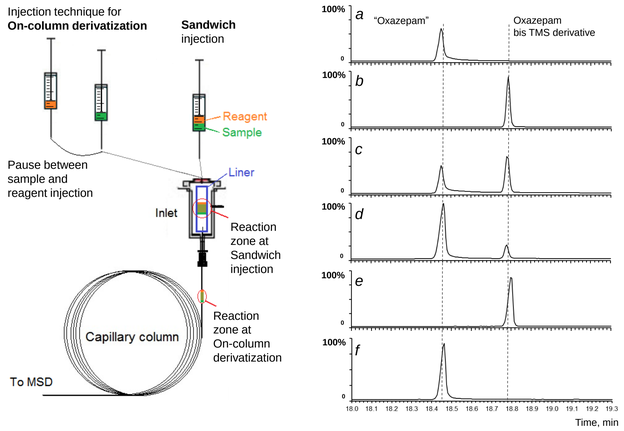
Open Probe Fast GC-MS: MS conditions were used as in the standard method, GC conditions: Rx1-5HT capillary column (2.0 m, 0.25 mm, 0.1 µm, ~ 2.0 mL/min He flow); oven temperature program – from 60°C to 320°C at 200°C/min; inlet and transfer line 220°C.

Fast GC-MS with Cold EI: GC-MS with Cold EI is based on a GC and MS interface with a supersonic molecular beam (SMB) and on the EI of sample compounds while they are vibrationally cold in the SMB (hence the name Cold EI) in a fly-through ion source. GC conditions: Rxi-1HT capillary column (15 m, 0.32 mm, 0.1 µm, 24 mL/min He flow); oven temperature program – from 80°C to 160°C at 40°C/min then to 300°C at 20°C/min; inlet temperature 240°C.

RESULTS and DISCUSSION

Oxazepam (R^4=H) and Lorazepam (R^4=Cl) under EI do not show peaks of molecular ions while in the spectra of their $N_{(1)}$-methyl analogs these peaks are present. Peaks of ions due to loss of water can be used for determination of $M^{+\cdot}$ of Oxazepam and Lorazepam. However, one can suggest that these molecules are converted to quinazolines in a GC injection port, and GC retention indices and the spectra recorded correspond to dehydration products. It also has been established earlier [1] that toluene or xylene solutions of Oxazepam and Lorazepam at 110°C decompose with the formation of corresponding quinazoline aldehydes; they are the products of water elimination.

GC retention index values and mass spectra indicate degradation most of Oxazepam and Lorazepam molecules in a GC injection port and further water elimination in a GC column, and probably in EI source (Figure 1). Additional experiments on “Open Probe Fast GC-MS” have confirmed degradation of Oxazepam and Lorazepam in a GC system (injection port and column), and experiments conducted on “Fast GC-MS with Cold EI” demonstrated sample degradations in the ion source as well.



a) Inlet temperature 200ºC, standard injection of the Oxazepam solution.

b) Inlet temperature 200ºC, standard injection of the Oxazepam bisTMS derivative synthesized prior GC-MS experiment.

c) Inlet temperature 150ºC, sequential injection of the Oxazepam sample and silylating reagent (MSTFA) - On-column derivatization.

d) Inlet temperature 200ºC, sequential injection of the Oxazepam sample and silylating reagent (MSTFA) - On-column derivatization.

e) Inlet temperature 200ºC, Sandwich injection of the Oxazepam sample (1mg/mL in acetonitrile) and silylating reagent (MSTFA).

f) Inlet temperature 200ºC, standard injection of the Oxazepam sample preheated at 200ºC followed by silylation with the MSTFA reagent.

Figure 1. Total ion currents (TIC) obtained for Oxazepam (***a***), products of its silylation carried out prior (***b***) and online GC-MS analysis(***c***, ***d***, ***e***), and for dehydration product of Oxazepam at 200ºC (***f***).

EI mass spectral data for the standard GC experiment, Open Probe and Cold EI

Mass spectral data acquired for Lorazepam on the traditional GC-MS (Figure 2***a***), on Open Probe Fast GC-MS (Figure 2***b***) and on Fast GC-MS with Cold EI (Figure 2***c***) demonstrate that we actually observe a spectrum of Quinazoline aldehyde (not Lorazepam) when spectrum is obtained on a traditional GC-MS instrument. A replacement of a regular GC with an Open Probe Fast GC-MS produces a spectrum that contains spectra of Quinazoline aldehyde and Lorazepam; a spectrum of an unseparated mixture of compounds is acquired. A spectrum of individual Lorazepam is obtained only on Fast GC-MS with Cold EI instrument. However, this spectrum cannot be considered as a traditional EI spectrum since low mass peaks are discriminated and intensities of molecular ions are elevated in Cold EI ion source spectra. The results presented in Figures 2 ***a***-***c*** establish that thermal decomposition of Lorazepam occurs in the inlet system and in the ion source.

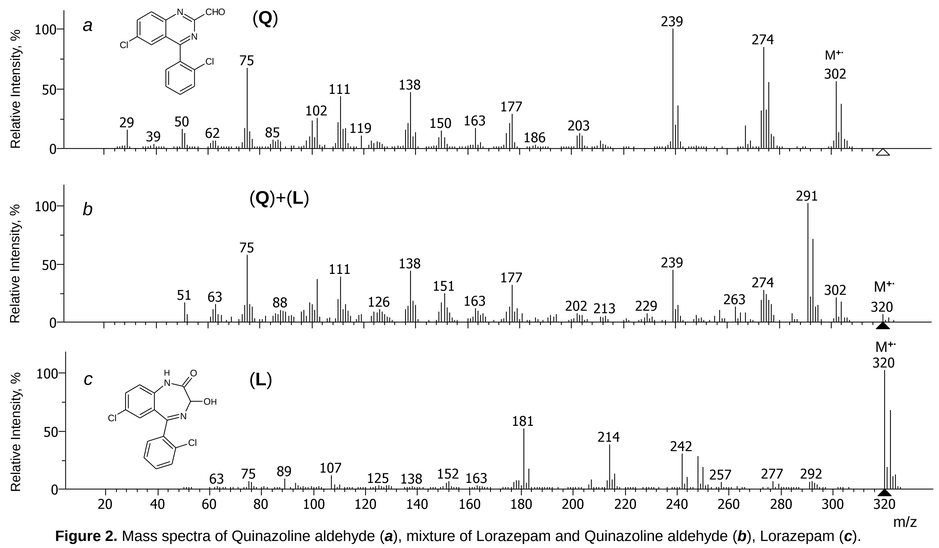
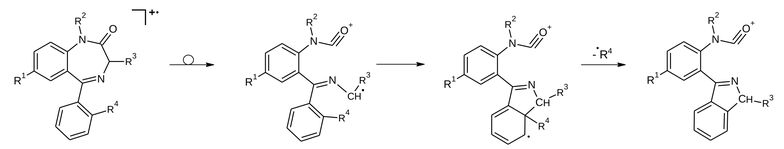


Figure 2. Mass spectra of Quinazoline aldehyde (***a***), mixture of Lorazepam and Quinazoline aldehyde (***b***), Lorazepam (***c***).

Substituents at 5-phenyl are the major donors of eliminating radicals under EI:



R^1 = Cl, NO_2; R^2 = H, $SiMe_3$; R^3 = H,$OSiMe_3$; R^4 = Cl, D, F, H

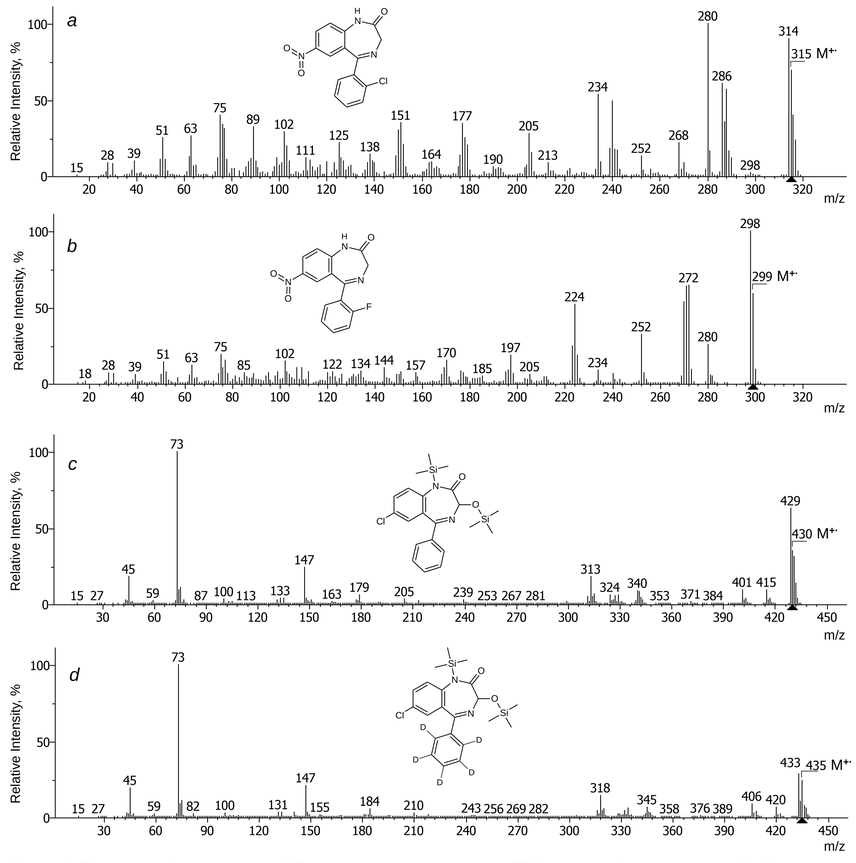


Figure 3. EI spectra of Clonazepam (***a***), N-Desmethylflunitrazepam (***b***) and TMS ethers of Oxazepam (***c***) and D_5-Oxazepam (***d***).

CONCLUSIONS

- Lorazepam and Oxazepam molecules undergo dehydrating processes in a GC system and in the ion source;
- Information on stability of Lorazepam and Oxazepam molecules and their transformation at different stages of GC-MS analysis is obtained and optimal trimethylsilylation conditions for their GC-MS analysis are established;
- An aromatic ring at C-5 is a major source for the elimination of hydrogen and halogen radicals;
- The Influence of a nature of $N_{(1)}$ on dissociative ionization of benzodiazepines is evaluated.

REFERENCES

[1] M.S. Siddegowda, et al. Indian Journal of Chemistry. 2012, V. 51B, P. 1628