

# Elucidation of molecular structure of bromazepam opened ring related compound detected in bromazepam tablets in acidic dissolution media using LC/MS

Viktorija Jakimovska Pokupec\*, Katerina Janchevska, Marija Grozdanovska, Viktorija Petrovska

Research and Development, Alkaloid AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

## Introduction

Bromazepam or 7-bromo-1,3-dihydro-5-(2'-pyridyl)-2H-1,4-benzodiazepin-2-one belongs to the 1,4-benzodiazepines group of drugs that are used in the treatment of anxiety, insomnia, seizures, muscle spasms and similar disorders. As found in literature, bromazepam in acidic conditions undergoes reversible reaction of 4,5-azomethine bond cleavage to the opened-ring compound formation. Having this in consideration, in the process of performing comparative dissolution profiles in media pH 1.2 and pH 4.5, bromazepam would undergo this reversible ring opening reaction (Damjanović et al., 2004).

During the analysis of comparative dissolution profiles in media pH 1.2 and pH 4.5 with an in house HPLC method for dissolution, two main peaks were detected, one originating from bromazepam and other with relative retention time (RRt) 0.72. In order to evaluate the nature of the compound with RRt 0.72, observed in dissolution media pH 1.2 and pH 4.5, comprehensive Liquid Chromatography / Mass spectrometry (LC/MS) analysis and detailed literature survey were conducted.

The aim of this study was to reveal the possible nature and fragmentation pathway of the compound with RRt 0.72 (Retention time about 1.51 min) from two manufacturers of bromazepam based finished products (bromazepam tablets) and a reference standard, using advanced analytical techniques such as MS. The study as well, is aimed to justify and demonstrate that the in house High Performance Liquid Chromatography (HPLC) method is also suitable for determination of open ring compound.

## Materials and methods

### Materials

Samples from three tablets strengths (1.5 mg; 3 mg; 6 mg) obtained from two manufacturers of bromazepam tablets were analyzed. Bromazepam certified reference standard (CRS) (batch number 4.0), obtained from European Pharmacopeia was also used. All samples were dissolved in pH 1.2 and pH 4.5 acidic dissolution media prepared according European Pharmacopeia. For the preparation of the mobile phase, CH<sub>3</sub>COONH<sub>4</sub>, glacial acetic acid and acetonitrile were used.

### Dissolution method

Dissolution test procedures was performed with standard apparatus II on Agilent 708-DS Dissolution apparatus. The procedure was conducted at 37°C, with 75 rpm in 900 mL volume of regulatory dissolution media pH 1.2 and pH 4.5, using sampling time point at 45 minutes.

### LC method

The analysis was performed on Thermo Scientific Dionex Ultimate 3000 UHPLC-UV-DAD system coupled with Thermo Scientific LTQ XL linear ion trap mass spectrometer. LC parameters were used according to in-house analytical procedure for dissolution of Bromazepam tables where 0.01M ammonium acetate buffer with pH 5.5 and acetonitrile in ratio 60:40 (v/v%) was used as a mobile phase. An analytical HPLC column, XBridge C18, 150 mm x 4.6 mm with 5 µm particles, at

temperature of 25°C with flow rate of 1.2 mL/min and injection volume of 20 µL from the sample and standard solutions were used. Bromazepam peak is obtained at about 2 minutes in a run time of 6 minutes.

#### MS method

MS ion source parameters were employed as follows: electro-spray ionization (ESI) was used, spray voltage was set at 5.0 kV, capillary temperature was set at 275 °C, sheet and auxiliary gas flows were 30 and 8 psi respectively. MS spectra were acquired by full range acquisition covering m/z 50-800, in positive ionization mode (Kozak et al., 2008). The instrument was controlled by Chromeleon 5.0 and Xcalibur 2.0 data analysis software. Mass Frontier spectral interpretation software 7.0 was used for the fragmentation pathway prediction and elucidation of the proposed structure of the unknown compound.

## Results and discussion

After dissolution of Bromazepam tablets in pH 1.2 and pH 4.5, the HPLC analysis showed peak of bromazepam on retention time 2.09 min (due to bromazepam) and unknown peak with retention time 1.51 min (RRt=0.72). The mass spectra of the peak occurring at Rt 2.09 min in all analyzed samples (in dissolution media pH 1.2 and in pH 4.5) showed protonated molecular ion at 316 m/z as parent ion of bromazepam (molecular weight, Mr 315 g/mol).

Following fragmentation ions were observed: 288 m/z, 184 m/z, 155 m/z, 127 m/z, 237 m/z, 261 m/z and 209/210 m/z. In accordance with the literature findings (Rivera et al., 2006; NIST database, 2014; Tas et al., 1986), these ions are characteristic fragmentation ions of bromazepam. The fragmentation mechanisms and characteristic fragments were confirmed using Mass Frontier spectral interpretation software 7.0.

As found in literature, bromazepam in acidic conditions (as in dissolution media pH 1.2 and pH 4.5) undergoes reversible reaction of 4,5-azomethine bond cleavage to the opened-ring compound formation, suggesting the possible origin of the unknown peak at Rt about 1.51 min (Damjanović et al., 2004). The mass spectra of the peak at Rt 1.51 min (RRt=0.72) revealed fragment ion at 334 m/z which corresponded to the protonated molecular weight of the structure of the 4,5-azomethine opened-ring compound (Mr=333 g/mol). Other fragment ions at 316 m/z, 288 m/z, 261 m/z, 237 m/z, 209 m/z, 184 m/z, characteristic for bromazepam fragmentation indicated a structure similar to bromazepam.

Comparable MS spectra and fragmentation patterns of the unknown peak were observed in bromazepam tablets from both manufacturers in all three tablet strengths and in both dissolution media. Furthermore, the findings were additionally confirmed using bromazepam CRS prepared to correspond with all three strengths as the finished product and in both dissolution media.

## Conclusion

Comparable MS spectra and fragmentation pathways of bromazepam and compound at Rt about 1.5 min were obtained when analyzing bromazepam CRS and bromazepam tablets manufactured by two manufacturers, in all dosage strengths and in both acidic dissolution media. The MS elucidation of the structure of the compound at RRt=0.72 confirmed that it is an opened-ring related compound of bromazepam. It can be concluded that this related compound could be detected in bromazepam tablets after acidic dissolution and it is originating from bromazepam due to molecular rearrangement (ring opening) in acidic conditions.

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## References

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