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Isolation and identification of a new impurity in lorazepam substance

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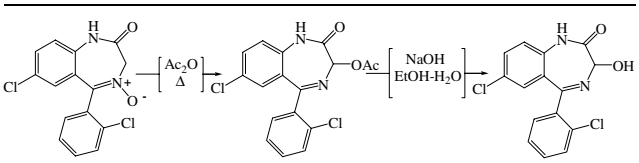
A new impurity in lorazepam (**1**) was UV-detected during the HPLC-analysis of compound **1**, batch scale. After the preparative chromatography on silica gel this impurity was isolated. According to IR, NMR, MS and elemental analysis the new impurity was identified as 7-chloro-5-(2-chlorophenyl)-2,3-dioxo-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (**2**).

1. Introduction

7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1*H*-1,4-benzodiazepine-2(3H)-one (**1**), well known under the generic name lorazepam has been produced by Polonovski rearrangement from 7-chloro-5-(2-chlorophenyl)-2*H*-1,4-benzodiazepine-2(3H)-one-4-oxide (**3**) followed by saponification of the resulting 3-acetoxy-7-chloro-5-(2-chlorophenyl)-1*H*-1,4-benzodiazepine-2(3H)-one (**4**), (Scheme 1) [1–3].

Scheme 1



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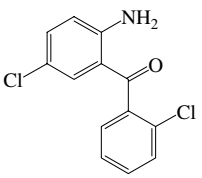
Well known and declared impurities in lorazepam (**1**) are: 2-amino-2′,5-dichlorobenzophenone (**5**), 3-acetoxy derivate **4** and 6-chloro-4-(2-chlorophenyl)-quinazoline-2-carboxaldehyde (**6**) [4]. Beside above-mentioned impurities USP Pharmacopoeia XXIV adduce other two possible impurities, 6-chloro-4-(2-chlorophenyl)-quinazoline-2-carboxylic acid (**7**) and 6-chloro-4-(2-chlorophenyl)-quinazoline-2-methanol (**8**), as products of disproportionation of aldehyde **6** [5].

Aminobenzophenone **5** is the starting material in the synthesis of compound **3** and product of hydrolytic cleavage of 1,4-benzodiazepine ring of any intermediate in the synthesis. 3-Acetoxy derivate **4** appears in traces from the saponification step (Scheme 1). Aldehyde **6** is the product of thermal decomposition of lorazepam (**1**) through dehydratation followed by ring contraction (Scheme 2).

These related substances in lorazepam (**1**) are, according to USP XXIV, examined by TLC in order to determine (semiquantitative) amount of impurities **4**, **5** and **6** [4]. Here we report on HPLC detection, isolation and identification of new impurity found in lorazepam substance.



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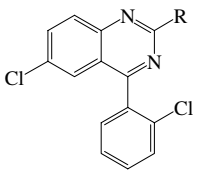
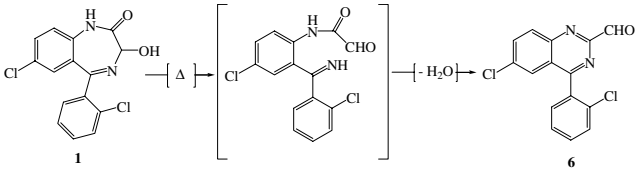


7: R = COOH
8: R = CH_2OH

Scheme 2



1 **6**

2. Investigations, results and discussion

During the HPLC analysis of lorazepam (**1**) active substance we observed an unsymmetrical peak. After change of chromatographic conditions we were able to separate a peak very close to that of lorazepam. It was obvious that the new impurity has very similar chromatographic properties, and on TLC despite many different eluents tested, has the same R_f value like lorazepam (**1**). In the same time we could achive minimal resolution of two peaks on HPLC (Fig. 1). Amount of new impurity was about 1.10% (HPLC-purity) in our lorazepam (**1**) batch.

Retention times in the obtained chromatogram showed that the new observed impurity had a retention time which differs from retention times of well-known and declared impurities: compound **4** – 8.43 min; compound **5** – 28.29 min; compound **6** – 11.02 min. In order to isolate the new impurity we decided to convert lorazepam (**1**) by thermal decomposition to aldehyde **6** (Scheme 2) in the hope that new impurity is thermally stable. Aldehyde **6** was separated by preparative chromatography, $R_f = 0.56$ with dichloromethane/2-propanol (9.5 : 0.5), and the new impurity identified as 7-chloro-5-(2-chlorophenyl)-2,3-dioxo-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (**2**) was isolated from the fractions with $R_f = 0.25$. Isolated compound **2** has the same retention time of 6.52 min than the new impurity (Fig. 2).

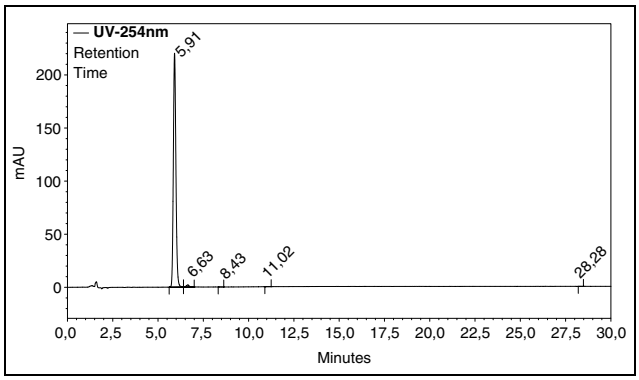


Fig. 1: HPLC chromatogram of lorazepam (**1**), r. t. = 5,91 min and new impurity **2**, r. t. = 6.63 min

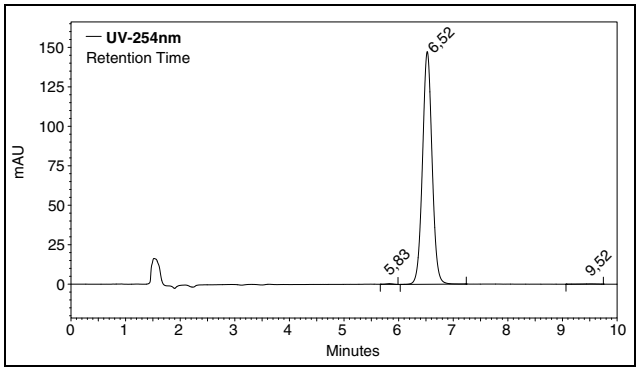
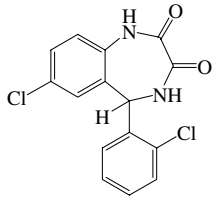


Fig. 2: HPLC chromatogram of new isolated and identified impurity **2**, r.t. (**2**) = 6.52 min

Nudelman et al. described preparation of compound **2** in low yield upon treatment of lorazepam with potassium hydroxide in THF/water mixture and characterised it by ^{1}H NMR spectra, elemental analysis and melting point [6]. This type of compound, namely 2,3-dioxo derivatives are well-known products of base-catalysed rearrangement of 3-hydroxy-1,4-benzodiazepines, oxazepam and temazepam [7–11]. However, to our knowledge, compound **2** was not recognized as possible impurity in lorazepam in official monographs [4, 12, 13].



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Structure of impurity **2** was based on IR, NMR, MS and elemental analysis. IR spectra of compound **2** shows a band of N-H's at 3230 and two very close bands of amide carbonyls at 1697 and 1681 cm^{-1}. From the ^{1}H NMR-spectrum of compound **2** a singlet originating from C-5 proton is present at 5.80 ppm and two singlets of N-H amide groups are present at 9.72 and 11.01 ppm. In the ^{13}C NMR spectrum of compound **2** there is a signal of benzylic C-5 carbon at 53.13 ppm and are two very close signals of amide-type carbon atoms at 162.12 and 163.12 ppm which also support structure **2**. From the COSY spectrum of compound **2** the interaction between benzylic proton and N-4 bonded proton is evident (Fig. 3).

Benzodiazepine **2** is probably formed during saponification of the 3-acetoxy derivate **4**, under the influence of hydroxide anion on already formed lorazepam, according to the previously described mechanism of base-catalysed rearrangement of oxazepam and temazepam [11]. The analyzed sample of lorazepam (batch scale) was obtained by saponification of compound **4** with 0.27 M sodium hydroxide in ethanol/water (46 : 54, V/V) at room temperature during 2 h. Despite the relatively mild reaction conditions, according to HPLC-analysis, compound **2** was formed in an amount of 1.10%. The same impurity was found in different batches of lorazepam. Prolonged saponification, during 96 hours causes an increase in content of compound **2** (approx. 36%, Fig. 4).

We have examined lorazepam tablets (Loram®) of one different producer (Lek, Ljubljana, Slovenia) by HPLC but compound **2** could not been detected.

After all, the question arises: how that up to now compound **2** was not detected in lorazepam active substance

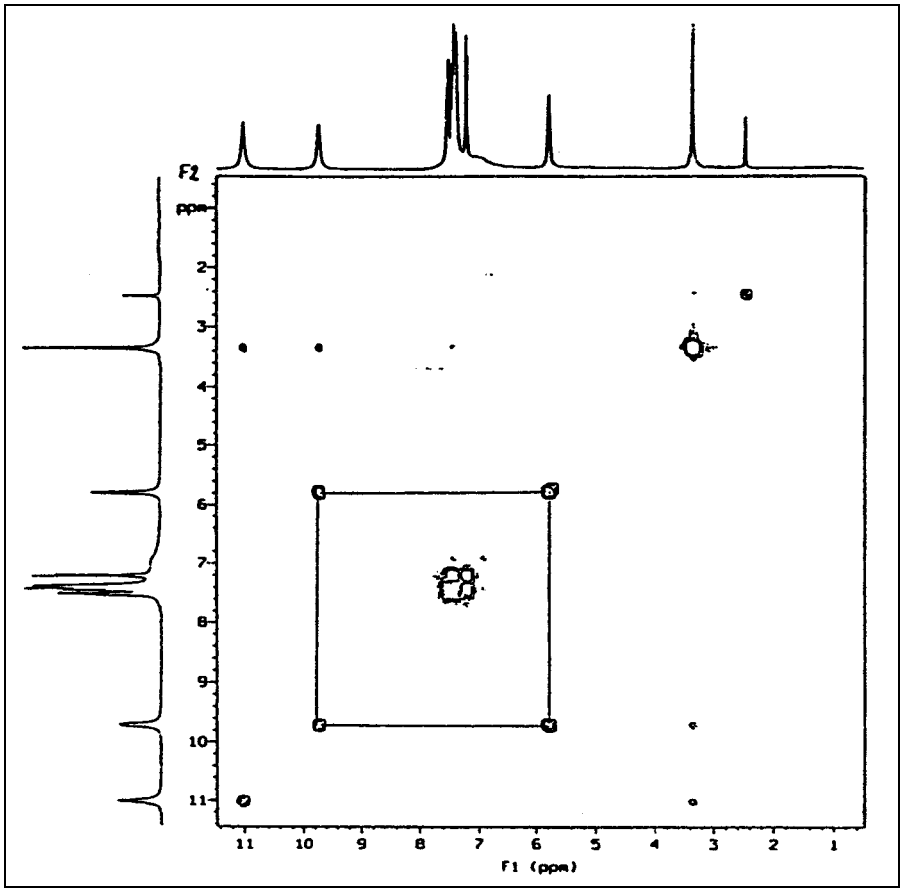

Fig. 3:
COSY-spectrum of compound **2** in DMSO-d$_6$

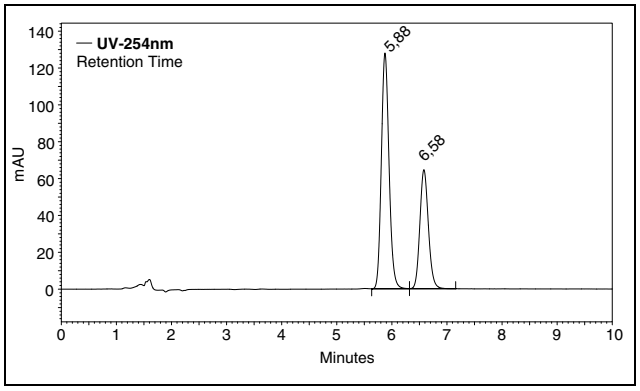

Fig. 4: HPLC-chromatogram of reaction mixture obtained by prolonged treatment of lorazepam with 0.27 M sodium hydroxyde in ethanol/water mixture (46 : 54, V/V)

and its pharmaceutical formulations? One answer which concerns lorazepam substance could be that official Pharmacopoeias (BP, USP, Ph.Eur) demand only TLC-analysis which is not selective enough to detect compound **2** due to a R_f value equal lorazepam. On the other side "prescribed" HPLC-analyses of related compounds in pharmaceutical formulations employ columns with particles of 5 μm. As we have employed a column of the latest technology and proven quality with particles of 3.5 μm in diameter, better selectivity between the two closest peaks, lorazepam and compound **2** together with a higher column efficiency is achieved. We consider that fact to be the main reason in separating and thereby detecting compound **2**. Further, some of the producers may have been using milder saponification methods in the last step of the synthesis and on that way "produce" only traces of compound **2**. In any case, analytical methods for quality control have to be re-evaluated.

3. Experimental

3.1. General notes

Lorazepam (**1**) and its 3-acetoxy derivate **4** was obtained from Belupo Pharmaceuticals & Cosmetics, batch scale (Koprivnica, Croatia). Compound **5** was purchased from Janssen Chimica (Beerse, Belgium), and compound **6** was prepared in our laboratory as previously described [14]. Solvents for reactions and preparative chromatography were purchased from Kemika (Zagreb, Croatia). Methanol and THF for HPLC were purchased from Fluka (Buchs, Switzerland). TLC analyses were carried out on silica gel sheets, 60 F_{254}, and preparative chromatography on silica gel, ϕ 0.063–0.2 mm, Merck (Darmstadt, Germany). NMR spectra were recorded on Varian (Palo Alto, CA, USA) XL-300 GEM (300 MHz) spectrometer, and IR spectra on a Perkin Elmer FT-IR Spectrum One instrument. MS analysis was carried on the Extrel 2000 FT-MS (ICR) instrument. HPLC analyses were carried out on a Thermo Separation Products system (San Jose, CA, USA) which consisted of a vacuum degaser SCM1000, pump P4000, autosampler AS3000, UV/VIS detector UV3000HR and Chrom Quest 2.51 software. Melting point was determined on Büchi's (Buchs, Switzerland) B-540 instrument. The HPLC method to obtain the resolution between lorazepam (**1**) and compound **2** was as follows – column: Waters (Milford, MA, USA) Symetry Shield RP-18 (150 × 4.6 mm; 3.5 μm), mobile phase: MeOH/H_2O/THF = 55 : 40 : 5 (V/V); flow: 1.0 ml/min; room temperature (24 °C); UV: 254 nm.

3.2. Isolation of 7-chloro-5-(2-chlorophenyl)-2,3-dioxo-2,3,4,5-tetra-hydro-1H-1,4-benzodiazepine (**2**)

A solution of lorazepam (**1**, 3.0 g, 9.34 mmol) was heated under reflux of 30 ml absolute EtOH under nitrogen atmosphere for 24 h. The reaction mixture was evaporated and dried in high vacuum. The resulting yellow viscous oil (2.95 g) was separated by preparative chromatography on silica gel (200 g) with CH_2Cl_2/2-PrOH (9.5 : 0.5) as eluent. Fractions with $R_f = 0.25$ were collected and evaporated. The obtained crystals (90 mg) were further purified by refluxing in 5 ml of 2-PrOH for 15 min. After cooling, crystals were separated by filtration and heated in 5 ml of toluene for 30 min. At the end crystals were filtered again and dried in high vacuum for several hours. 55 mg (1.83%) of benzodiazepine **2** were obtained as colorless crystals, m. p. 329.5–330.5 °C (lit. m.p. = 330–331 °C [6]).
HPLC–purity of benzodiazepine **2**: 99.5%, r. t. = 6.52 min, Fig. 2.
IR (KBr) ν: 3230, 3140, 3092, 2926, 1697, 1681, 1597, 1575, 1486, 1469, 1447, 1307, 1281, 1217, 1204, 1188, 1168, 1126, 1099, 1081, 1054, 1043, 944, 928, 895, 875, 848, 803, 752, 727, 712, 693, 670, 643, 613. ^{1}H NMR (DMSO-d_6) δ: 5.80 (s, 1 H, CH), 7.21 (d, 1 H, J = 8.6 Hz, arom.), 7.40–7.54 (m, 6 H, arom.), 9.72 (s, 1 H, CHNH), 11.01 (s, 1 H, NHCO).
^{13}C NMR (DMSO-d_6) δ: 53.13, 123.84, 127.44, 128.78, 129.10, 129.32, 130.34, 130.62, 133.03, 134.49, 135.48, 162.12 (CHNHCO), 163.12 (NHCO). MS m/z $(M + H)^+$ = 322 (23%); m/z $(M - H)^+$ = 320 (29%); m/z $(M-CO)^+$ = 293 (9%); m/z $(M-COCONH)^+$ = 250 (26%); m/z $(250\text{-}2\,H)^+$ = 248 (44%); m/z $(248\text{-}Cl)^+$ = 214 (70%); m/z $(293\text{-}C_6H_4Cl-H)^+$ = 181 (100%)
Elemental analysis: Calc: w(C) = 56.10%, w(H) = 3.14%, w(N) = 8.72%. Found: w(C) = 56.19%, w(H) = 3.18%, w(N) = 8.79%.

3.3. Base-catalysed rearrangement of lorazepam (**1**)

To the suspension of lorazepam (1.61 g, 5 mmol) in 16 ml of 96% EtOH, a solution of sodium hydroxide (0.40 g, 10 mmol, 2 eq) in 20 ml of distilled water was added with stirring. Reaction mixture was stirred for 96 h. Then acetic acid (1 ml) was added and crystals were filtered and washed with 3 × 20 ml of distilled water. After drying in high vacuum for several hours 1.48 g of product was obtained as colourless crystals. HPLC analysis showed that the product contained ca. 36% of compound **2** and 64% of unconverted lorazepam.

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