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In-process control of midazolam synthesis by HPLC

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Abstract

A high-performance liquid chromatographic assay coupled with UV detection (239 nm) has been developed for the determination of midazolam and its synthesis precursors. The separation of the analytes was performed on a Kromasil C_8 column (15 cm × 4.6 mm i.d., 5 μm) at 30 °C. The mobile phase [ammonium chloride (pH 5.5, 1 g l^{-1})–methanol–acetonitrile (45:22:33, v/v/v)] was pumped at a flow-rate of 1.5 ml min^{-1}. This method is rapid (less than 11 min), sensitive (limit of detection (LOD) ranged between 0.05 and 0.5 mg l^{-1}) and selective for the determination of midazolam, and it could be used for monitoring different synthetic routes.

Keywords: Midazolam; In-process control; HPLC

1. Introduction

In the history of psychoactive drugs, the discovery of chlordiazepoxide by L.H. Sternbach and L.O. Randall in 1957 represents a milestone. This compound was the origin of a large number of therapeutically useful compounds within the class of benzodiazepines. Although the name benzodiazepine has become almost synonymous with anxiolytic, the members of this series all show, to a various degree, other properties such as anticonvulsant, muscle relaxant and sleep-inducing activity, combined with excellent tolerance.

Midazolam is 8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine and belongs to a newer class of benzodiazepine derivatives [1]. The presence of an imidazole ring in the 1,2-position induces some changes in the properties characteristic of classical benzodiazepines such as basicity, stability in aqueous solution and rate of metabolism [2]. It is widely used in anesthesia because of its relatively short elimination half-life (2–5 h) and for sedation of artificially ventilated patients in intensive care units. Besides, midazolam has anticonvulsant properties [3].

Several synthetic routes have been developed for midazolam synthesis, and high-performance liquid chromatography (HPLC) analysis is essential for monitoring, and differentiate from the corresponding synthesis intermediates, these production process.

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The analytical techniques published for midazolam, and eventually some of its metabolites, have mainly involved HPLC [4–15], gas chromatography (GC) [16,17], GC–MS [18–20], or even radioimmunoassay [21]. To the date, there are no publications concerning the simultaneous analysis of midazolam and its synthetic intermediates and potential impurities, and it is well known that a well functioning process monitoring system is necessary in the optimization of purification and production processes, and to maintain the conditions at the optimal level required to secure production of high purity midazolam with maximum yield. In our experience, the desired yield and purity can only be reached through improved and expanded analytical control, where reversed-phase chromatography (RP-HPLC) has a central role.

This paper describes a rapid and reliable isocratic HPLC method for the determination of midazolam and its synthesis intermediates. The utility of the system is demonstrated with the development of applications of industrial significance. Chromatograms of actual samples from in-process control of midazolam are discussed.

2. Experimental

2.1. Reference compounds

Reference substances midazolam, 6-chloro-2-chloromethyl-4-(2-fluorophenyl)-1,2-dihydroquinazoline 3-oxide (Compound **1**), 6-chloro-2-chloromethyl-4-(2-fluorophenyl) quinazoline 3-oxide (Compound **2**), 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2-nitromethylene-2*H*-1,4-benzodiazepine 4-oxide (Compound **3**), 2-aminomethyl-7-chloro-2,3-dihydro-5-(2-fluorophenyl)-1*H*-1,4-benzodiazepine dimaleate (Compound **4**), 8-chloro-3a,4-dihydro-6-(2-fluorophenyl)-1-methyl-3*H*-imidazo[1,5-*a*][1,4]benzodiazepine (Compound **5**), 2-(α-bromoacylamino)-5-chloro-2′-fluorobenzophenone (Compound **6**), 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (Compound **7**), 7-chloro-5-(2-fluorophenyl)-2-methylamino-3*H*-1,4-benzodiazepine (Compound **8**), 7-chloro-5-(2-fluorophenyl)-2-(*N*-nitrosomethylamino)-3*H*-1,4-benzodiazepine (Compound **9**), and 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2-nitromethylene-2*H*-1,4-benzodiazepine (Compound **10**) were kindly supplied by Asturpharma S. A. (Asturias, Spain). Benzophenone was purchased from Fabbrica Italiana Sintetici S.p.A. (Vicenza, Italy).

2.2. Chemicals

Ammonium chloride for analysis was supplied by Merck (Darmstadt, Germany).

Acetonitrile and methanol, both of gradient grade quality were purchased from Panreac (Barcelona, Spain).

The water used in the mobile phase preparation was first distilled and then deionized in a Milli-Q apparatus (Millipore, Bedford, MA, USA).

2.3. Standard solutions

Standard solutions of midazolam and its intermediates were prepared at a concentration of 1 g l^{-1} by dissolving the appropriate amount of the drug in the mobile phase. Dilutions of the 1 g l^{-1} standards were used to make the appropriate working solutions of the drugs.

2.4. Sample preparation

The liquid samples for process control of the different reaction steps were pipetted (25 μl) accurately into 50 ml volumetric flasks and subsequently diluted to volume with mobile phase.

The solutions were sonicated in an ultrasonic bath for 1 min and filtered through a 0.45 μm PTFE syringe filter (Análisis Vínicos, Tomelloso, Spain) and then 20 μl were injected into the HPLC apparatus.

2.5. Chromatography

The chromatographic system consisted of two LC-10ADvp chromatographic pumps, an SIL-10ADvp autoinjector, and an SPD-M10Avp diode array detector which were all coupled by a programmable system controller SCL-10Avp (Shi-

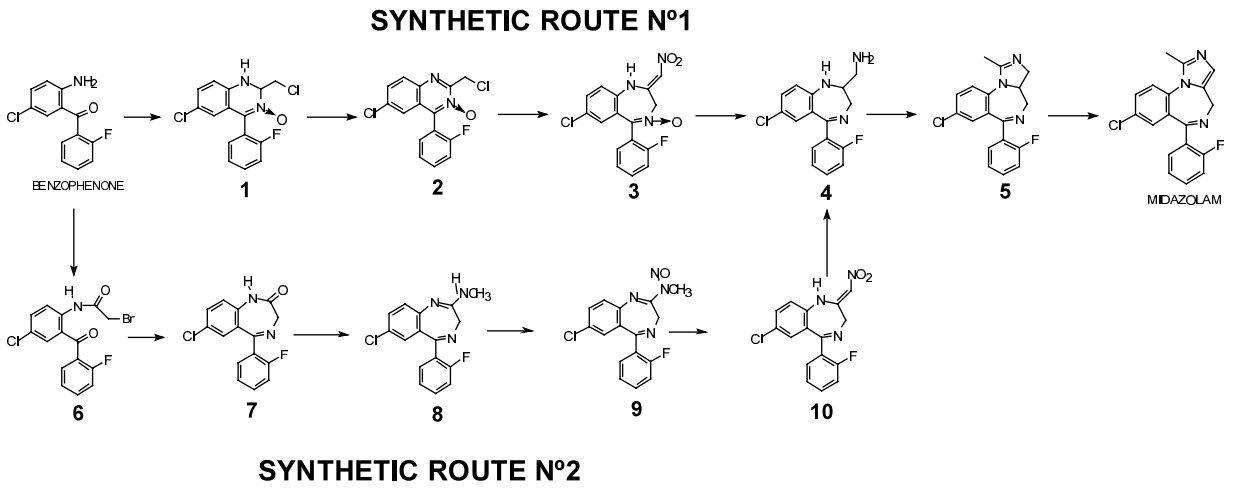

Fig. 1. Synthetic route of midazolam. Chemical structure for the compounds referred to in the text.

madzu, Columbia, MD, USA). Detection took place at 239 nm.

During method development, two prepacked (150 × 4.6 mm i.d., 5 μm particle size) columns were tested: Kromasil C_8 and C_{18}, both purchased from Análisis Vínicos (Tomelloso, Spain).

The column temperature was maintained at 30 °C with the aid of a model MFE-01 temperature controller (Análisis Vínicos, Tomelloso, Spain).

The mobile phase consisted of ammonium chloride (pH 5.5, 1 g l^{-1})–methanol–acetonitrile (45:22:33, v/v/v). The final solution was filtered through a 0.45 μm Nylon pore filter (Análisis Vínicos, Tomelloso, Spain) and degassed prior to use. The mobile phase was pumped at a flow-rate of 1.5 ml min^{-1}.

3. Results and discussion

Among the reported synthesis of midazolam, two are the most common procedures. These routes, outlined in Fig. 1 have been tested at Asturpharma's factory to develop an industrial scale method for its production.

In this context, for the optimization of the different key variables in the midazolam production, it was necessary to develop an analytical method suitable for identifying and quantifying the different synthetic intermediates and for analyzing the purity of the final product. Based on its properties, HPLC was the analytical tool chosen to carry out our assays.

For the chromatographic assay of the final product midazolam, different chromatographic methods were proposed [4–15]. In our case, these methods did not prove to be successful. During method development, the nature of the stationary phase was evaluated by using Kromasil C_8 and C_{18} chromatographic columns. Kromasil C_8 gave the best results according to peak symmetry and resolution.

Different pH and ionic strength were investigated. Potassium phosphate, ammonium acetate, ammonium chloride were employed in this study in order to fit the pH between 5 and 9.3 and ionic strength up to 130 mmol l^{-1}. Below pH 5.5 compounds **4** and **5** are partially overlapped and its peak profile is not good. Between pH 5.5 and 9.3 all the compounds are well separated. Ionic strength above 5 mmol l^{-1} obtained by using ammonium chloride gave the best results. In consequence, pH 5.5 and 1 g l^{-1} ammonium chloride was selected for subsequent experiences.

The type and content of the organic modifier were also checked. Acetonitrile was discarded because it provides poor band spacing. Compounds **4** and **5** are partially overlapped and moreover, midazolam and compound **3** coeluted.

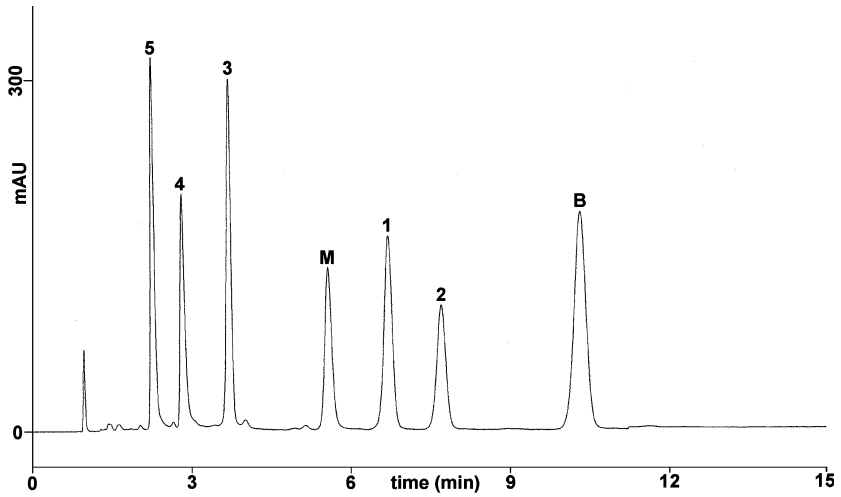

Fig. 2. Chromatogram of a mixture of standards corresponding to midazolam and its synthetic intermediates (Route 1). Chromatographic conditions as reported in Section 2.5. Peak identification: 5, Compound **5** (35 mg l^{-1}); 4, Compound **4** (26 mg l^{-1}); 3, Compound **3** (33 mg l^{-1}); M, midazolam (30 mg l^{-1}); 1, Compound **1** (37 mg l^{-1}); 2, Compound **2** (22 mg l^{-1}); B, benzophenone (36 mg l^{-1}).

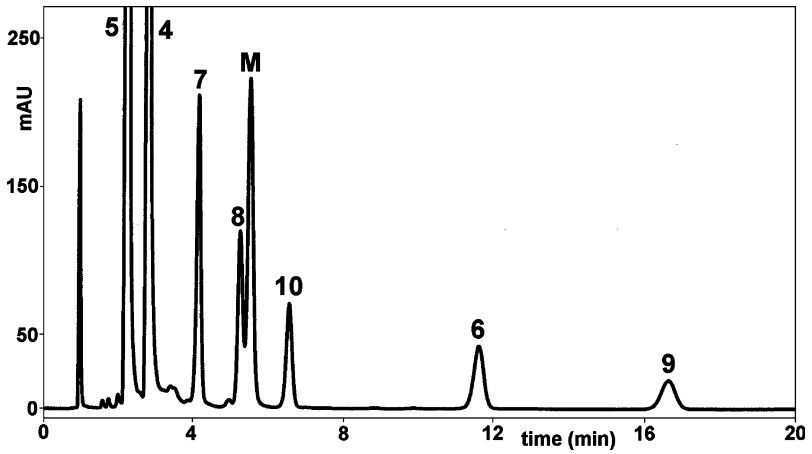

Fig. 3. Chromatogram of a mixture of standards corresponding to midazolam and its synthetic intermediates (Route 2). Chromatographic conditions as reported in Section 2.5. Peak identification: M, midazolam. The rest of the peaks corresponds to those of Fig. 1.

Significant changes in reverse-phase selectivities were observed on changing the organic modifier from acetonitrile to methanol. Nevertheless, the solvent strength of methanol was too low for compounds **1** and **2**, midazolam and benzophenone, and the analysis time was too large. In addition, peak tailing were observed for some compounds. With the purpose of taking advantage of the acetonitrile solvent strength and the methanol selectivity a ternary mobile phase was selected as the optimum: ammonium chloride (pH 5.5, 1 g l^{-1})–methanol–acetonitrile (45:22:33, v/v/v).

In order to give shorter analysis times, the flow-rate of the mobile phase was set to 1.5 ml min^{-1}.

Table 1
Analytical characteristics for the chromatographic determination of midazolam and its precursors (Route 1)

	Retention time (min)	Slope	Intercept	R^2	LOD (mg l^{-1})	Within-run precision (RSD%)	Recoveries (%)
Midazolam	5.5	0.9126	0.042	0.9995	0.2	1.4	99.3–100.7
Compound **1**	6.7	0.8036	−0.1508	0.9999	0.1	0.9	99.5–102.1
Compound **2**	7.7	0.9971	−0.1345	0.9999	0.1	1.4	95.9–100.5
Compound **3**	3.7	0.9238	−0.353	0.9996	0.2	0.7	96.6–100.9
Compound **4**	2.8	0.7398	0.4565	0.9995	0.5	1.5	96.4–105
Compound **5**	2.3	0.7562	0.3107	0.999	0.03	1.0	96.6–102.7
Benzophenone	10.3	1.3401	0.1227	0.9993	0.05	0.4	99.5–101

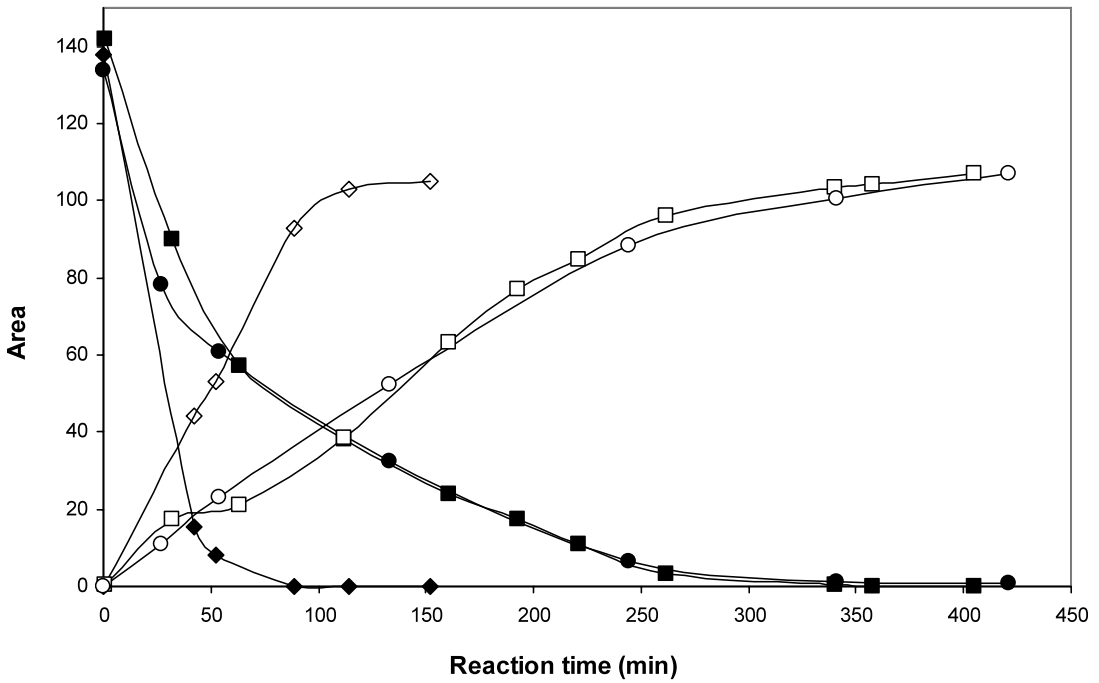

Fig. 4. Influence of catalysts on the synthesis of compound **5**. (□, ■) Without catalyst, (◆, ◇) *p*-toluene sulphonic acid, and (●, ○) dimethylaminopyridine. Empty figures represent reaction product (Compound **5**) and filled figures show the evolution of the starting material (Compound **4**).

In Figs. 2 and 3, chromatograms of midazolam and its corresponding synthesis precursors from both synthetic routes are presented. Experimental results showed a good peak symmetry and resolution for all the analytes in each procedure. Only a partial overlapping between midazolam and compound **8** was observed for synthetic route 2 (Fig. 3).

From a industrial view point, route number 1 resulted more adequate in terms of yield and simplicity. In consequence, our efforts have been directed to the optimization of this synthetic procedure.

3.1. Analytical characteristics

The values presented in Table 1 for limit of detection (LOD), precision and recoveries show the good performance of the analysis of midazolam and its intermediates from route 1.

The LOD was determined by injecting serial dilutions of a concentrated standard mixture,

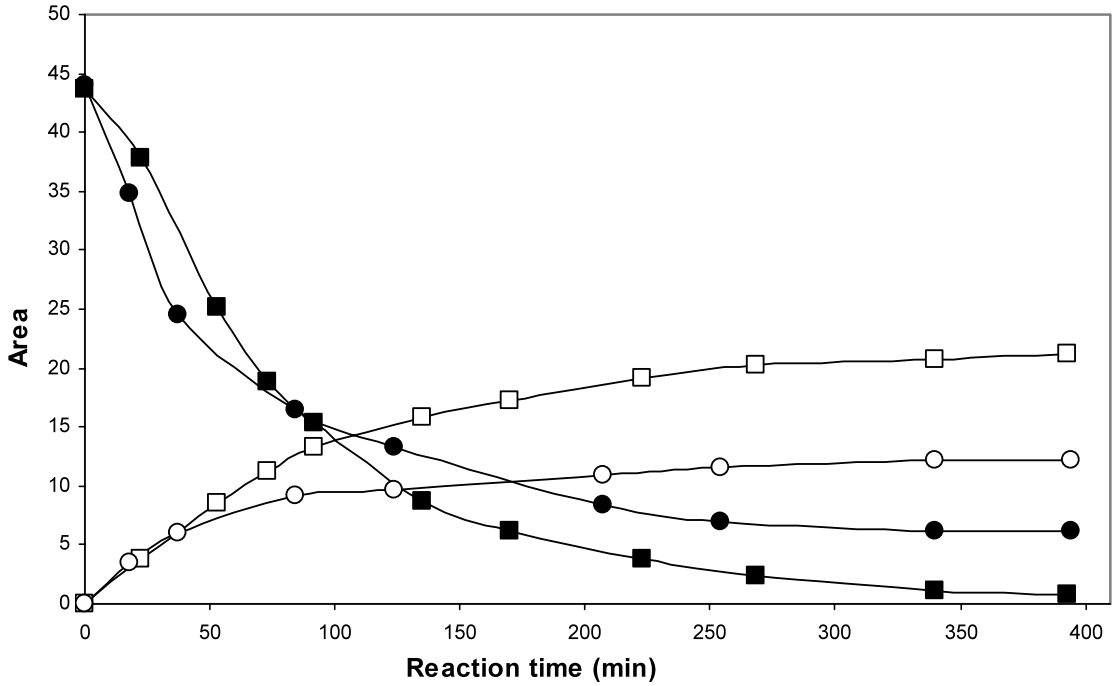

Fig. 5. Influence of the oxidation agent (MnO_2) on the synthesis of midazolam. (□, ■) Process carried out with commercial activated MnO_2. (●, ○) Process carried out with homemade γ-MnO_2. Empty figures represent reaction product (midazolam) and filled figures show the evolution of starting material (Compound **5**).

followed by the preparation of calibration plots, which were extrapolated to a signal-to-noise ratio (S/N) of 3 so as to assign the detection limit (according to Winefordner and Long [22]).

Within-run precision was obtained by replicate analyses ($n = 11$) of one mixture of midazolam and its precursors on the same day. As can be seen, satisfactory results were obtained for all compounds. These range from 0.4 to 1.5% relative standard deviation (RSD) at mid-calibration range.

The determination of percentage recovery was calculated by comparing the absolute response of the processed (recovered) substances to the absolute response of the external standards. The recovery of the midazolam was determined in triplicate at mid-calibration range. The recoveries were 99.3–100.7%, testifying to the accuracy of the proposed method.

The linearity range was among the quantification limit and at least up to 120 mg l^{-1} for all the compounds, except for the compound **4** (206 mg l^{-1}). All the compounds gave a linear correlation coefficient over the studied range greater than 0.999, showing good linear proportionality between concentration and detector response. The linearity of the calibration graphs was checked with the lack-of-fit statistical test. This test evaluates the variance of the residual values [23]. The calculated values were lower than the tabulated ones ($\alpha = 0.01$), linearity thus being demonstrated.

3.2. In-process control

This chromatographic method has been successfully employed to monitorize the influence of different variables (time, temperature, use of catalysts, etc.) in several key steps of the midazolam synthesis. These processes are relatively clean reactions with few by-products at small quantities ($< 2\%$) that do not interfere with the HPLC analysis. The reaction solvent (toluene in both cases) do not interfere because it elutes between midazolam and compound **2**.

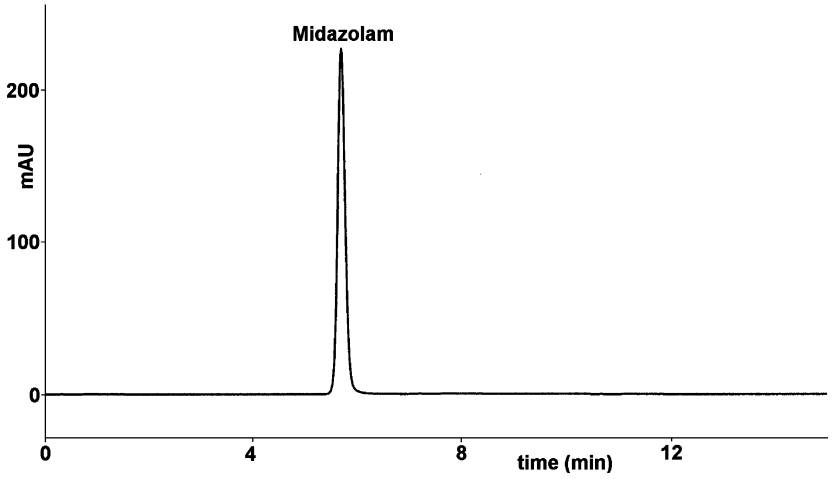

Fig. 6. Chromatogram of a sample of the final product (midazolam) obtained following the route 1. Chromatographic conditions as reported in Section 2.5.

Fig. 4 shows the influence of catalysts on the condensation of the two amino groups of intermediate **4** with triethyl orthoacetate to give the imidazoline **5**. As can be seen, dimethylaminopyridine is not a good catalyst. In this reaction, the *p*-toluene sulphonic acid is an efficient catalytic agent because when it is added to the reaction, the kinetic of the reaction is highly increased (the reaction concludes four times faster than without catalyst).

Subsequently, the final synthetic step, that is a critical step of the synthetic procedure, was studied (Fig. 5) in order to choose the most appropriate oxidation agent. Two different MnO_2 were evaluated (commercial activated and homemade [24] γ-MnO_2). In our experiments, commercial MnO_2 was selected because using γ-MnO_2 gives poorer results in terms of conversion and reaction rates (approximately twice lower).

Fig. 6 shows that midazolam obtained following this synthetic route has a great purity and no significant amount of by-products is observed.

4. Conclusions

The utility of the system is demonstrated with the development of applications of industrial significance such as the analysis of midazolam and its synthetic intermediates for in-process control of midazolam synthesis.

The described method yielded good selectivities and precision. In addition, the LOD was in the range of the low mg l^{-1}, and the analysis time is short.

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