Mediterranean Journal of Pharmacy & Pharmaceutical Sciences
www.medjpps.com

ISSN: 2789-1895 online
ISSN: 2958-3101 print

SHORT COMMUINCATION article

A comparative study of intravenous midazolam marketed in Libya

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Received: 12-09-2024, **Revised:** 28-10-2024, **Accepted:** 06-11-2024, **Published:** 31-12-2024

HOW TO CITE THIS
Belaid et al. (2024) A comparative study of intravenous midazolam marketed in Libya.
Mediterr J Pharm Pharm Sci. 4 (4): 9-14. [Article number: 175]. https://doi.org/10.5281/zenodo.14042921

Keywords: Assay, benzodiazepine, chromatographic, Infrared, Pharmacopeia

Abstract: Midazolam, a benzodiazepine medication, is used for sedation during diagnostic and therapeutic medical procedures. Insufficient doses of sedatives, including midazolam, can result in patient anxiety and awareness during the procedure. Several brands of midazolam are available in the Libyan market. This study aims to identify and estimate the medication content of intravenous midazolam in various marketed products using different analytical methods. Product identity was confirmed using Infrared methods (IR) and retention times of High-Performance Liquid Chromatographic methods (HPLC). Quantification was performed using a rapid reverse-phase HPLC method. Chromatographic analysis was conducted on a C18 column (250 mm×3.3 mm I.D., 5.0 µm particle size) with a mobile phase comprising acetonitrile, methanol, and 0.065 M ammonium acetate buffer (50: 20: 30, v/v/v), adjusted to a pH of 5.5±0.02 with orthophosphoric acid, at a flow rate of 1.0 ml/min. Ultraviolet (UV) detection was set at 220 nm. The identification results met British Pharmacopeia (BP) standards. However, the midazolam content in the Tunisian brand was shallow compared to the products from Germany and Switzerland. Thus, post-marketing testing is essential to assess the quality of critical drugs like midazolam, and further investigations, including clinical evaluations and regulatory follow-up, are necessary.

Introduction

Benzodiazepines exert their effects on the central nervous system (CNS) by interacting with $GABA_A$ receptors, which are distributed across various regions of the brain. Midazolam (MDZ) a hydrophilic benzodiazepine, is highly soluble in water, contributing to its rapid onset and short duration of action. As part of the benzodiazepine class (**Figure 1**), MDZ induces a range of effects, including anxiety relief, sedation, muscle relaxation, hypnosis, and anticonvulsant activity. Compared to barbiturates, which can lead to coma when administered in high doses, benzodiazepines are considered safer [1, 2]. MDZ's binding to $GABA_A$ receptors in the CNS produces an inhibitory effect, leading to its sedative and amnesic properties. With a short duration of action (1.5-3.5 hrs), it is widely used as an anesthetic agent, particularly in intensive care units [3]. As noted earlier, MDZ (**Figure 2**) is a member of the benzodiazepine family, known for its favorable efficacy and safety profile. This highlights the importance of chemical, pharmacokinetic, and clinical studies of this class of compounds [4, 5]. MDZ was first synthesized by Hoffmann-La Roche Laboratories in 1976, exhibiting modified properties compared to

ISSN: 2789-1895 online
ISSN: 2958-3101 print

classical benzodiazepines [6]. Incorporating a fused imidazole ring endows MDZ with increased basicity and hydrophilicity, along with enhanced stability against metabolic degradation. The nitrogen atom in the imidazole ring is primarily responsible for the basicity of MDZ, with a pKa of 6.15, enabling the formation of water-soluble chloride and maleate salts. Consequently, MDZ can be formulated for parenteral administration, allowing for intramuscular or intravenous delivery [6, 7].

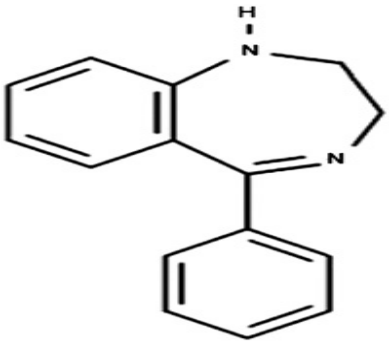

Figure 1: General structure of benzodiazepines

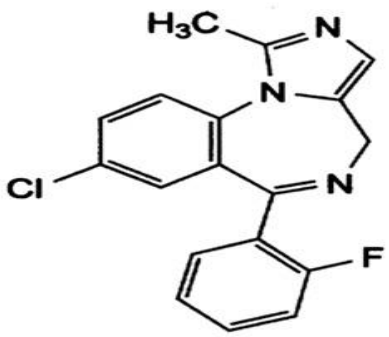

Figure 2: Chemical structure of midazolam

Unlike other benzodiazepines, MDZ demonstrates high stability against hydrolysis. Its rapid onset of action can be attributed to its fast metabolic inactivation, facilitated by the methyl group at position one on the fused imidazole ring [8]. Because of its precision, accuracy, and sensitivity; liquid chromatography, and especially HPLC, is widely applicable for MDZ quantification and its metabolites in biological matrices of humans, rats, and rabbits' plasma [9-11], as well as methods to evaluate the quality of pharmaceutical formulations, compiled in the review [12]. This study involves the identification of MDZ using the IR scanning method, along with quantification via HPLC. The drugs tested are listed in **Table 1**.

Table 1: Origin of the three drugs tested

Samples	Origin
A	Switzerland
B	Germany
C	Tunisia

Material and methods

Infrared (IR) spectroscopy: Liquid samples were extracted to obtain clearer IR spectra, and the spectra were recorded using an IR spectrometer (Shimadzu, Japan). The extraction procedure was as follows:

1. 15.0 mg of samples B and C were weighed into separate beakers.
2. A few milliliters of 5.0 M ammonia were added to each sample.
3. The mixture was transferred to a separatory funnel.
4. For each sample, 7.5 mL of dichloromethane was added, and the mixture was shaken and allowed to separate.
5. 7.5 mL of dichloromethane was also added, shaken again, and separated.
6. Sodium sulfate was used to dry the extract.
7. The samples were filtered using a Buchner funnel.
8. The filtrate was dried in an oven at 50°C.

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ISSN: 2958-3101 print

The IR absorption spectrum for MDZ was recorded using a KBr disc (1.0 mg of the substance mixed with 200 mg KBr) on an IR spectrometer (Bruker, USA). Infrared spectroscopy data revealed that IR spectra for samples A, B, and C were recorded over the range of 4000 to 500 cm^{-1}. The findings are shown in **Figures 3, 4,** and **5**.

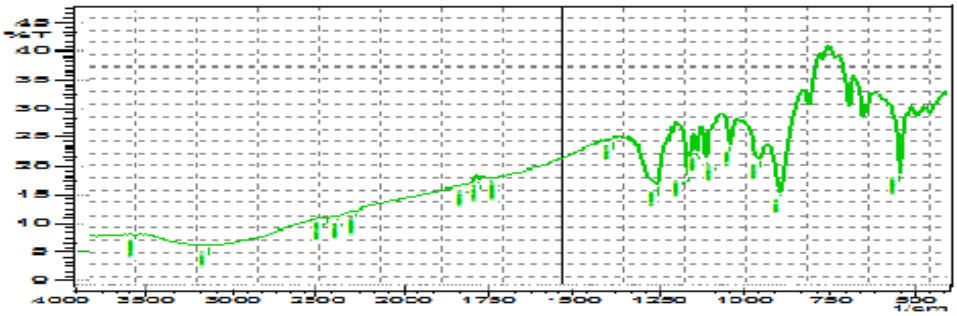

Figure 3: IR spectra of sample A

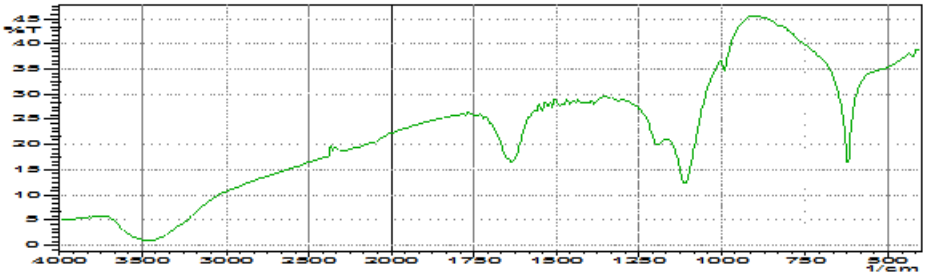

Figure 4: IR spectra of sample B

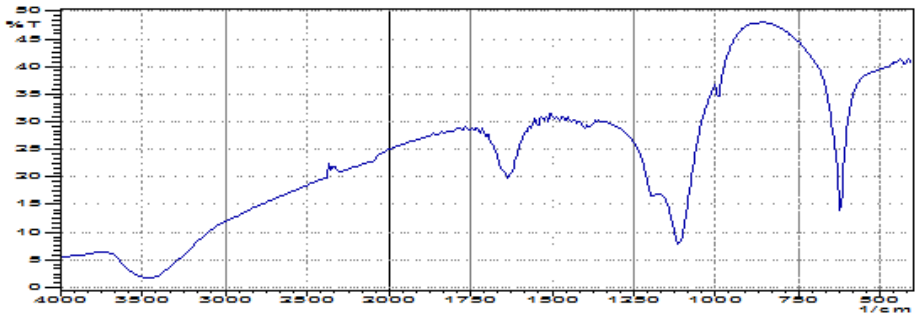

Figure 5: IR spectra of sample C

The IR spectra revealed characteristic peaks corresponding to functional groups, as summarized in **Table 2**. Differences in spectra suggested variability in the excipient composition across the brands, potentially contributing to inconsistencies in drug content.

Table 2: Location of some functional groups of midazolam on IR spectra

Peak assignment	Wave number cm^{-1}
C=O	1090
C=N	1611
C-H from the imidazolic heterocycle	3200-3500

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ISSN: 2958-3101 print

High-Performance Liquid Chromatographic Methods (HPLC analysis): In this study, HPLC analysis was performed using a UFLC system (Shimadzu, Japan). The chemicals used, including orthophosphoric acid and methanol, were supplied by Carlo Erba Reagents (France), and triethylamine was also utilized. These reagents were critical in ensuring the precise measurement of MDZ concentrations across different products [9].

Chromatographic conditions: The method for MDZ assay was adapted from BP 2008 guidelines using HPLC (UFLC, Shimadzu, Japan). The analysis utilized an SPD-20AV detector, with a mobile phase consisting of 77:28 (v/v) methanol and an equal volume mixture of 0.1 M orthophosphoric acid and 0.03 M trimethylamine. Separation was carried out on an Ascentis C18 column with dimensions of 250 mm length and 4.6 mm internal diameter. The flow rate of the mobile phase was maintained at 1.0 ml/min, with the oven set to 40°C, and detection was performed at a wavelength of 220 nm. The injection volume was 3.0 µl. Due to the absence of a reference standard, product A was utilized as the reference for comparison with the other two products [9].

Sample preparation: Each ampoule contained 5.0 mg/ml of MDZ. For preparation, the content of three ampoules was mixed (15 mg in total), and a volume equal to 10 mg of each product was transferred into a 50 ml volumetric flask, and the mobile phase was added to reach a final volume of 50 ml. This will prepare a solution containing 0.01%w/v of MDZ. The resulting solutions were filtered using a Pall 0.2 µm GHP Acrodisc 13 mm filter and injected into the HPLC apparatus for analysis [9].

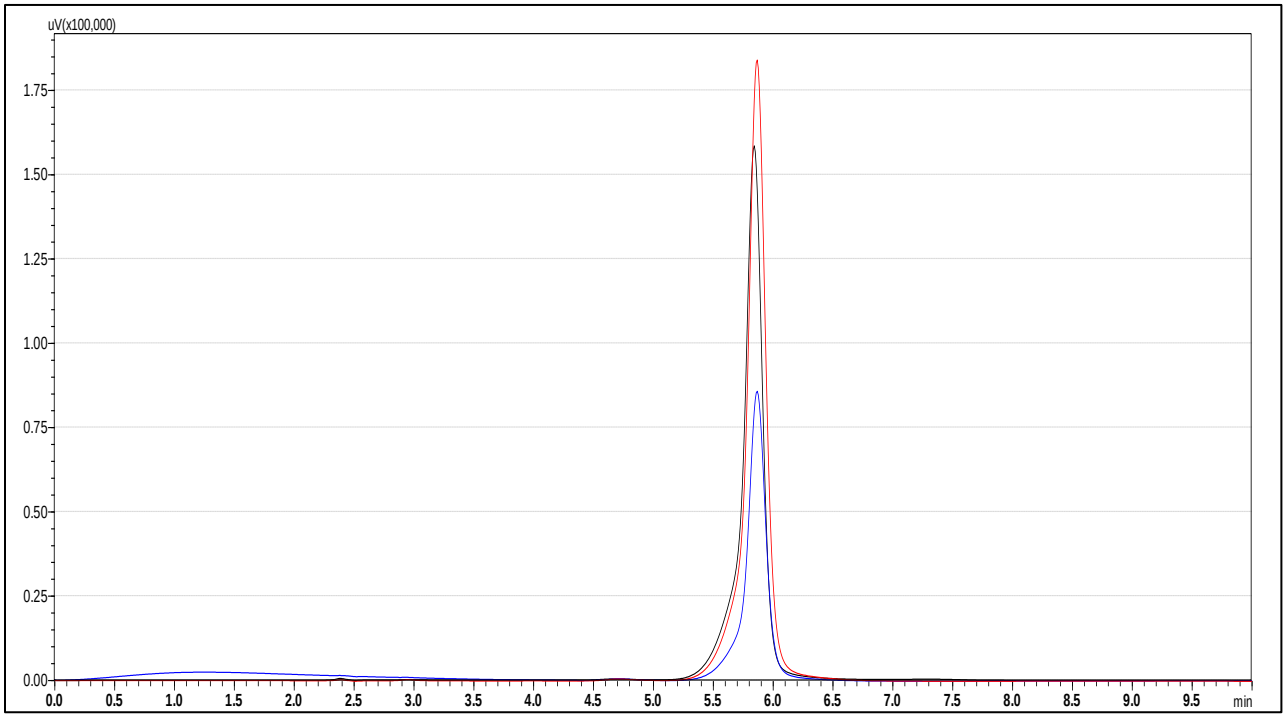

Figure 6: HPLC data (midazolam chromatogram); Red: A; **Black**: B; **Blue**: C.

Figure 6 shows the HPLC chromatograms for the three MDZ products, identified by the following color codes: Red: Product A, Black: Product B and Blue: Product C. In all chromatograms, a peak at approximately 5.8 min (retention time) corresponds to MDZ, confirming its identity in products B, and C, relative to the reference product A. The peak areas were used to calculate the percentage recovery of MDZ in the injections. The content of MDZ in product B was found to be 86.6%, while product C had a significantly lower content of 46.5% when compared to product A, **Table 3**.

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Table 3: Chromatographic data for A, B and C products

Product	Retention time (min)	Area	% recovery
A	5.8	2181439	Reference
B	5.7	1889937	86.6%
C	5.8	1015042	46.5%

Discussion

As product A was sourced from a reliable manufacturer, the results for products B and C were compared to it. The identification results for all three products were comparable, with similar IR spectra, and retention times, suggesting that the chemical identities of the drugs were alike [11, 12]. However, more investigations for possibilities of degradation or the presence of impurities are needed especially with the appearance of the small peak at 1.5 min observed with product C, **Figure 6**, and BP [12]. These impurities may have risen from substandard manufacturing processes or inappropriate storage or handling and therefore need to be studied thoroughly according to ICH guidelines [13, 14]. The chromatographic data showed similarities in the chemical structure of the main compound in the three products. However, the variation in peak areas and heights indicated that product C had significantly lower MDZ content. These stark differences highlight the need for testing multiple batches from the same manufacturer to ensure consistent quality. Appropriate actions, such as stricter regulation or potential withdrawal of certain batches, should be considered if further testing shows substandard content.

Conclusion: Midazolam (Product C) is widely used in Libyan clinics, despite the significant discrepancy in drug content. Considering these results, further investigations into different batches are essential along with different chemical methods of analysis and clinical studies. Moreover, feedback reports must be provided to regulatory authorities to ensure quality control management, and subsequently, stricter measures may be necessary for pharmaceutical products that do not meet acceptable standards, including potential withdrawal from the market.

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Author contribution: AKB &WEE conceived and designed the study. AKB, ZAE, WEE & RAG collected and analyzed data. AKB, RMK & ZAE performed data analysis and interpretation. AKB & RMK drafted and revised the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for its contents.
Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: Including plagiarism, informed consent, data fabrication or falsification, and double publication or submission were completely observed by the authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.