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ORIGINAL ARTICLE

Optimization of the Yields in the Synthesis of Midazolam

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ABSTRACT

Midazolam is a short-acting benzodiazepine used extensively for sedation and anaesthesia. Conventional synthetic pathways tend to produce varied yields and purities. The current work aimed to optimise the oxidation and purification steps to improve reproducibility, yield, and purity. The objective of this study was to compare the different oxidation and purification techniques used in midazolam synthesis and determine the optimal trial for producing high-purity products by analytical validation. Midazolam was prepared in two stages. Step I involved the condensation of 2-aminomethyl-7-[2-fluorophenyl]-1H-1,4-benzodiazepine dimaleate with triethylorthoacetate in a biphasic medium. Stage II was oxidation using MnO_2 obtained by three procedures (X, Y, Z) and purified using methods (P, Q, R). The products were characterised by TLC (Rf ~0.62), IR spectroscopy, 1H NMR, mass spectrometry (m/z 325), melting point analysis, and high-performance liquid chromatography (HPLC). Trial 8 (Method Z for MnO_2 and Method R for purification) yielded a maximum crude yield (92.10%) and pure midazolam base (74.72%) with 99.96% purity. The spectral data established the structure: $C_{18}H_{13}ClN_3F$, MW 325.7 g/mol, and MP 158°C. The combination of MnO_2 (Method Z) and purification (Method R) is a reproducible and high-yield method for midazolam synthesis. This method improved the purification of midazolam with high efficiency and product purity.

Keywords: Midazolam; Manganese dioxide oxidation; HPLC; Analytical methods

INTRODUCTION

Midazolam, a benzodiazepine with short action, is used extensively in clinical practice for its anxiolytic, sedative, and amnestic actions.[1] The synthesis of chlordiazepoxide by L.H.Sternbach and L.O.Randall, in 1957, was a landmark in the development of psychoactive drugs.[2] This drug has given rise to numerous therapeutically beneficial benzodiazepines. Although commonly linked with anxiolytic activity, benzodiazepines have anticonvulsant, muscle relaxant, and sleep-promoting actions with favourable tolerance. Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,3] benzodiazepine), is a more recent class of benzodiazepine derivatives.[4] Its 1,2-imidazole ring alters standard benzodiazepine properties, affecting basicity, aqueous stability, and metabolic rate.[3] It is widely applied in anaesthesia because of its short elimination half-life (2–5 h) and is also used to sedate ventilated intensive care patients. Midazolam further has anticonvulsant activity.[5]

Its synthesis has been widely investigated owing to its important therapeutic value, requiring effective and high-yielding production processes.[5] The chemical structure of Midazolam encompasses a fused benzodiazepine and imidazole ring system, whose assembly comprises crucial oxidation and purification steps with strong impacts upon overall yield and purity.[3] Synthesis of Midazolam most typically incorporates the oxidation reactions, whose selection of oxidizing agent can have huge impacts upon reaction efficiency and by-product formation.[6] Optimization of these conditions, however, is still a challenge owing to the fine balance between the completion of the reaction and minimisation of degradation.

Purification methods, such as recrystallisation, chromatography, and solvent extraction, are also essential to produce a product that passes pharmaceutical-grade purity requirements. Analytical validation techniques like high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy are invaluable tools for determining product purity and establishing struc-

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tural integrity.[7,8] The aim of this study was to systematically examine different methods of oxidation and purification for the synthesis of midazolam in order to find the most effective trial that yields high-purity midazolam. Through the use of thorough analytical validation, this research aims to develop better synthetic protocols that will be easily applicable for industrial-scale production, with consistency and quality in pharmaceutical use.

MATERIALS AND METHODS

All reagents and chemicals used in this study were of Analytical Reagent (AR) and Laboratory Reagent (LR) grades and were obtained from reputed vendors, such as Lancaster, Nice, Sigma, Spectrum, NR Chem, Rolex, S. D. Fine-Chem Ltd., and Merck. Important chemicals involved were acetone, manganese sulfate, chloroform, methanol, cinnamyl alcohol, nitric acid, dichloromethane, potassium permanganate, ethyl acetate, silver nitrate, hexane, sodium hydroxide, hydrochloric acid, sodium sulfate, isopropanol, toluene, maleic acid, triethylorthoacetate (TEOA), manganese dichloride tetrahydrate, and xylene. The synthesized products were analyzed with various analytical methods: the melting points were established using Thiel's tube method for purity determination; thin-layer chromatography (TLC) utilized silica gel plates with mobile phases like dichloromethane: methanol (4.8:0.2) and hexane:ethyl acetate (7.5:2.5), spots viewed under UV light for determination of Rf values; infrared (IR) spectra were obtained through the KBr pellet method on a SHIMADZU FTIR 8700S spectrophotometer between 4000–400 cm^{-1} for identifying functional groups; nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Spectrospin-400 MHz instrument using chloroform as solvent and tetramethylsilane (TMS) as internal reference, recorded at IISc and AstraZeneca Pharma, Bangalore; and mass spectrometry was carried out by direct injection on a Thermo Finnigan LCQ Deca XP Max spectrophotometer for measurement of molecular weights and fragmentation patterns.

For purity determination, an HPLC method was established using a SHIMADZU 10 ATVP pump assembly with a C8 column and a mobile phase consisting of ammonium acetate buffer and methanol. The stock and solution of midazolam were prepared by dissolving 5 mg of the active ingredient in methanol, sonicated, placed into the system, and monitored for detection at 254 nm. Midazolam stage-I synthesis was performed in three different ways (A, B, and C), all of which involved the reaction of 2-aminomethyl-7-[2-fluorophenyl]-1H-1,4-benzodiazepine dimaleate with triethylorthoacetate (TEOA) under biphasic conditions, reflux, and purification.

In Method A, the reaction was carried out in a water-dichloromethane (DCM) mixture under cold conditions (below 10°C), with pH adjusted using aqueous ammonia. The organic layer was separated, washed to neutrality, dried over sodium sulfate, and evaporated by vacuum distillation. The residue was redissolved in xylene and refluxed with TEOA, and the precipitated product was isolated by filtration, washed, dried, and characterised. Purification entailed dissolving impure midazolam in hot ethyl acetate, treatment with activated charcoal, filtering, concentration, and recrystallisation to give a product of practical yield of about 69-86% based on the method, melting points close to 145°C, and TLC Rf of 0.50–0.52. Method B employed an identical biphasic system with fresh and recycled xylene, and yielded similar yields after purification. Method C used a biphasic solvent consisting of water and toluene, topped with chloroform washes to eliminate oily impurities, reflux with TEOA, and purification similar to Method A, yielding lower yields (~33%). Stage II synthesis involves the oxidation of midazolam stage I using manganese dioxide (MnO_2) synthesised by different methods (X, Y, Z).

The reactions were usually refluxed in toluene for 4-16 h, followed by TLC monitoring, and the crude oxidised product was collected by filtration and removal of the solvent. Several trials used MnO_2 synthesized from potassium permanganate with activated charcoal (Method X), manganese dichloride tetrahydrate with $KMnO_4$ (Method Y), and manganese sulfate with sodium hydroxide (Method Z). Raw midazolam base was purified by precipitating either the hydrochloride salt with isopropanol-HCl or maleate salt with maleic acid, followed by pH adjustment with ammonia to obtain the pure base. Acetone, ethyl acetate, isopropanol, and hexane were used as solvents for purification. The yields were different in different trials, with the purity verified by TLC (Rf approximately 0.62–0.65), the melting points in the range of 155–160°C, and the high purity verified by IR, NMR, and mass spectrometry. The MnO_2 drying time and water content affected the product yield and oxidation efficiency, with increased drying time decreasing water content and increasing MnO_2 activity. The highest yields were achieved when MnO_2 was dried thoroughly, and the oxidation reactions were refluxed for a sufficient time. The purified midazolam base was routinely characterised using IR and 1H NMR spectroscopy to confirm the functional groups and molecular structures, as anticipated.

RESULTS

The results of several trials using different routes of manganese dioxide (MnO_2) synthesis for the oxidation of Midazolam Stage-I followed by purification were routinely performed and examined. The amount of compound used in Stage I and Stage II varied from 14 g to 100 g. Crude Midazolam base yield ranged from 65.09% to 92.10%, while the purity was from 71.64% to 87.00%, varying with the method of preparation of MnO_2 used (X, Y, or Z). Various solvent methods P, Q, and R were used for purification, which greatly improved the purity and produced the pure

base in quantities from 2.5 grams to 73 grams. The yield after purification ranged from 19.20% to 74.72%, whereas the purity of the purified midazolam base was significantly enhanced, with values up to 99.96%. The MnO_2 utilised in this trial was synthesised via Method Z, and purification was performed via Method R, both of which showed consistent and stable performance in other high-yielding trials. Trial 8 was found to be the best candidate, followed by Trial 7 according to the results. Trial 8 produced a maximum crude base content of 92.10%, with a crude purity of 96.35%. Purification resulted in the maximum amount of pure base obtained at 74.72%, along with an extremely high purity of 99.96%, and Trial 7 gave a pure base of 65.50% with an extremely high purity of 99.97% (Table 1).

The analytical identification of these compounds was established by Thiel's tube method melting point determination, TLC with hexane:ethyl acetate and dichloromethane: methanol solvent systems, IR using a SHIMADZU FTIR 8400S spectrometer, proton NMR spectra on a Bruker Spectrospin-400 MHz instrument with $CDCl_3$ as solvent, and mass spectrometry by electron spray ionization, all establishing the purity and identity of the synthesized Midazolam compounds.

The best yielding synthesis of Midazolam, yielded a pure product of molecular formula $C_{18}H_{13}ClN_3F$ and molecular weight 325.7 g/mol. The purified midazolam base isolated in this experiment had a melting point of 158°C, which was in close agreement with the literature values for pure midazolam. The theoretical yield for the synthesis was determined to be 97.71 g, whereas the practical yield was 73 g, with an excellent yield of 74.72%. Thin-layer chromatography (TLC) was carried out with a solvent system of dichloromethane: methanol (4.8:0.2), and the compound showed an Rf value of 0.62, which was good resolution and mobility.

Infrared (IR) spectroscopy also showed the presence of characteristic functional groups, and significant absorption peaks were found to be at 3075.29 cm^{-1} (aromatic C–H stretch), 2990.42 cm^{-1} (aliphatic C–H stretch), 1611.41 cm^{-1} (C=N stretch), 1486.05 cm^{-1} (C=C stretch), 1310.54 cm^{-1} (C–N stretch), 1214.11 cm^{-1} (C–F stretch), and 769.54 cm^{-1} (C–Cl stretch) (Figure 1).

Mass spectrometric analysis revealed a molecular ion peak at m/z 325 (M^+), further confirming the molecular integrity of the synthesised compound (Figure 2).

The proton NMR (^1H NMR) spectrum, recorded using $CDCl_3$ as the solvent, showed multiple peaks: a multiplet at δ 6.993–7.642 ppm for aromatic protons (7H), a singlet at δ 6.926 ppm for a proton on a methine group (CH), two doublets at δ 5.105–5.137 ppm and δ 4.024–4.062 ppm corresponding to methylene protons (CH_2), and a singlet at δ 2.553 ppm for the methyl group (CH_3, 3H) (Figure 3).

Figure 4 shows the HPLC chromatogram of the crude midazolam base from the Trial-8. The main peak appeared at

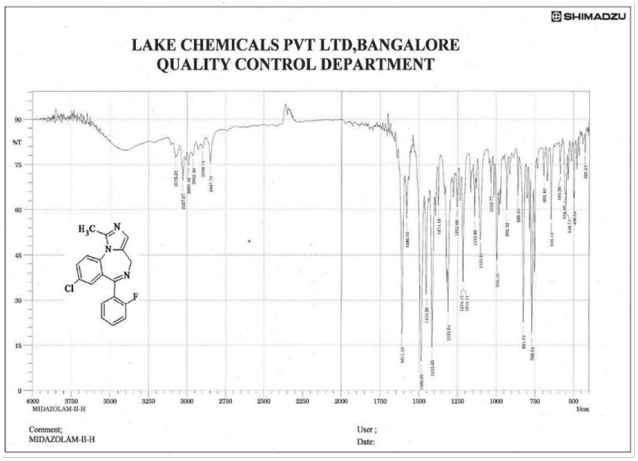

Fig. 1: IR Spectra of Pure Midazolam base (Trial 8)

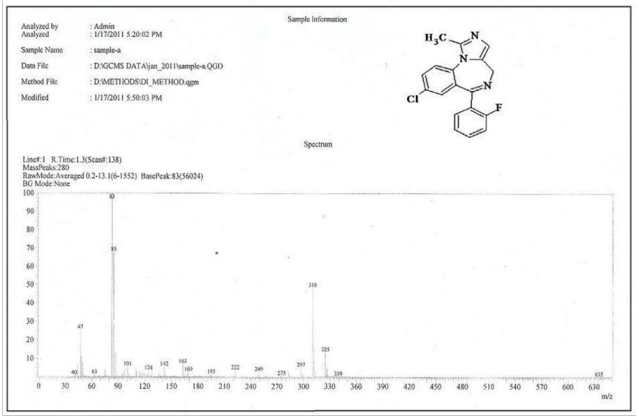

Fig. 2: Mass Spectra of Pure Midazolam base (Trial 8)

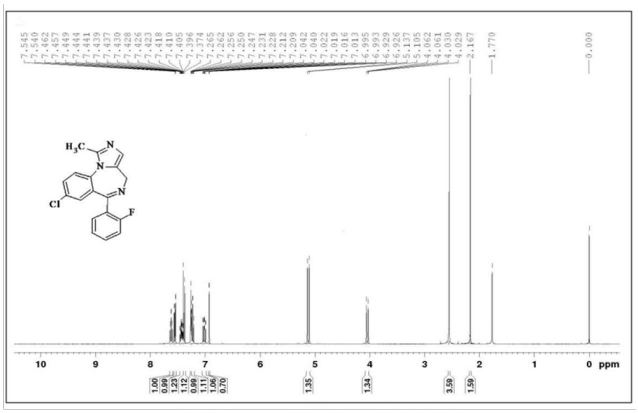

Fig. 3: NMR Spectra of Pure Midazolam base (Trial 8)

Table 1: Trials Showing % Yield and % Purity of Crude and Pure Midazolam Compounds Using Various MnO_2 Preparation and Purification Methods

Trial No.	Preparation of MnO_2 method	Stage 1 (g)	Crude base			Purification Method	Pure base (g)		
			g	% Yield	% Purity		g	% Yield	% Purity
1	X	16	10.0	76.80	65.09	P	2.5	19.20	80.41
2	Y	15	9.5	72.96	86.99	Q	4	30.72	96.99
3	Z	100	70.0	71.64	89.93	R	43	44.00	95.39
4	Z	100	74.0	75.73	93.29	R	50	51.17	96.67
5	Y	14	10.5	80.65	94.20	R	5	38.40	98.12
6	Z	100	77.0	78.80	94.99	Q	55	56.29	99.88
7	Z	100	85.0	87.00	95.65	P	64	65.50	99.97
8	Z	100	90.0	92.10	96.35	R	73	74.72	99.96

16.795 minutes with a 100.00% area, confirming the presence of a midazolam base. A minor impurity peak was observed at 8.730 min, contributing only 0.43% of the area.

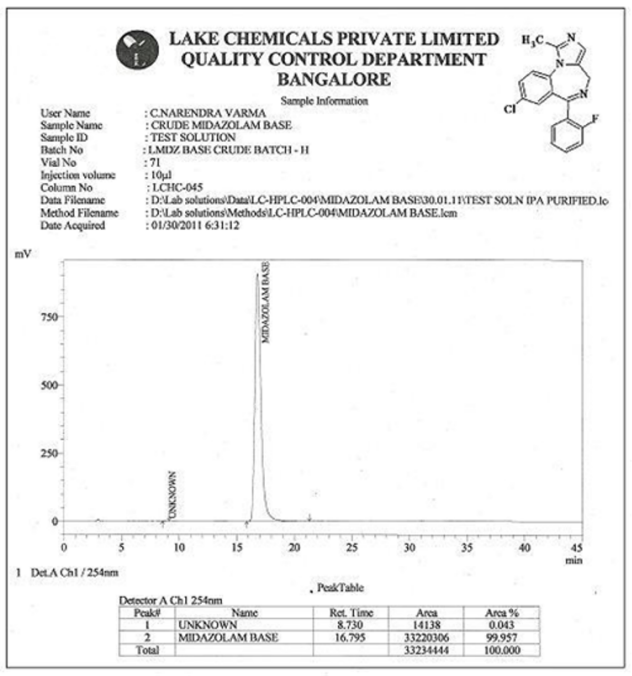

Fig. 4: HPLC of Pure Midazolam base (Trial 8)

DISCUSSION

Optimising the yield and purity of midazolam during synthesis is an important area of study in the bulk drug market. Finding efficient purification techniques is important to obtain a very pure drug with few impurities and fewer side effects. Solid organic products from reactions are not pure and contain trace amounts of impurities in addition to the desired drug. The purification of these impurities is typically achieved through crystallisation using appropriate solvents or solvent mixtures.

The current study systematically compared different procedures for the preparation of manganese dioxide (MnO_2) and their subsequent purification methods in midazolam synthesis. The crude midazolam base yield ranged between 65.09% and 92.10%, whereas the purity ranged between 71.64% and 87.00%, based on the MnO_2 preparation method used (X, Y, or Z). Purification was achieved through various solvent techniques, designated as P, Q, and R, which greatly improved the purity and produced a pure base in quantities between 2.5 and 73 grams. The percentage yield after purification ranged from 19.20% to 74.72%, whereas the purity of the purified midazolam base significantly increased, with values up to 99.96%.

Trial 8 was the most successful, giving the highest crude base yield (92.10%) with a crude purity of 96.35%. After purification, the highest yield of pure base (74.72%) and purity of 99.96% were obtained. The MnO_2 for this trial was prepared through Method Z and purified through Method R, both having consistent and steady performance for other high-yielding trials. These findings are consistent with other research findings, highlighting the significance of MnO_2 preparation routes in terms of oxidation efficiency and product yield. A patent from Teva Pharmaceutical Industries Ltd. details a method for preparing highly pure midazolam and its salts, emphasising the importance of purification methods in acquiring high levels of purity.[9] Overall, the combination of Method Z of preparing MnO_2 and Method R of purification was the most effective approach to synthesizing high-purity Midazolam, surpassing conventional methods reported in previous research.

CONCLUSION

Different MnO_2 preparation and purification processes were evaluated to determine the most reliable method. The comparison of different methods showed that Method Z for oxidation and Method R for purification gave high-purity midazolam bases in all trials. Trial 8 was the best, as it gave the maximum crude and pure yields with very high purity values. Thin layer chromatography (TLC), infrared

(IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry were used to establish the structural integrity and high purity of the resulting compounds.

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