European Journal of Chemistry

View Journal Online
View Article Online

Ultra-performance liquid chromatography determination of related compounds of molindone in drug substances

Balaji Nagarajan [1,*] Gunasekar Manoharan [1], Ganapathy Narayanan Shanmugam [1], Nataraj Palaniyappan [2] and Abhinav Yarragunta [3]

[1] *New Jersey Bioscience Centre, 675 US Highway 1, North Brunswick, New Jersey, 08902, United States*
[2] *Shri Jagdishprasad Jhabarmal Tibrewala University, Rajasthan, 333001, India*
[3] *Stanton College Preparatory School is a preparatory high school in Jacksonville, Florida, United States*

* *Corresponding author at: New Jersey Bioscience Centre, 675 US Highway 1, North Brunswick, New Jersey, 08902, United States.*
e-mail: balajin@rkpharmainc.com (B. Nagarajan).

RESEARCH ARTICLE

10.5155/eurjchem.13.2.180-185.2252

Received: 09 March 2022
Received in revised form: 16 April 2022
Accepted: 20 April 2022
Published online: 30 June 2022
Printed: 30 June 2022

KEYWORDS

UPLC
Molindone
Separations
Pharmaceuticals
Chromatography
Related compounds

ABSTRACT

Effective chromatographic separation was achieved on a phenyl-hexyl stationary phase (50×2.1 mm, 1.9 micron particles) with the economical and straightforward mobile phase combination delivered in isocratic mode at a flow rate of 0.6 mL/min at 254 nm using a ultra-performance liquid chromatography (UPLC) system. In the developed method, the resolution between molindone and its related compounds was more significant than 2.0. Regression analysis shows an r^2 value (correlation coefficient) greater than 0.999 for molindone and its associated compounds. This method could detect related compounds of molindone at a level below 0.009% with respect to a test concentration of 500 µg/mL for a 2.0 µL injection volume. The method has shown good, consistent recoveries for related compounds (90-110%). The test solution was found to be stable in the diluent for 48 hours. The drug was subjected to stress conditions. The mass balance was found to be close to 99.3%.

Cite this: *Eur. J. Chem.* **2022**, *13(2)*, 180-185 **Journal website**: www.eurjchem.com

1. Introduction

Molindone is an antipsychotic which is used in the United States in the treatment of schizophrenia. It works by blocking the effects of dopamine in the brain, leading to diminished symptoms of psychosis. It is rapidly absorbed when orally, and its molecular formula is $C_{16}H_{24}N_2O_2$. Molindone is a white to off-white or pale-pink crystalline powder. It is freely soluble in water and alcohol. Molindone was discontinued by its original supplier, Endo Pharmaceuticals, in 2010. The structures of related compounds and molindone is given in Figure 1 [1-3].

Several methods have been developed to determine molindone by HPLC and LCMS techniques [4-7]. The methods of references [4-7] explain the determination of molindone using HPLC, HPLC-MS, and HPLC-MS/MS techniques in plasma is tabulated in Table 1.

This research article describes a simple, sensitive, and cost-effective mobile phase method for the determination/quantitation of related compounds of molindone in drug substances. The work also includes method development and the complete validation [8] as per ICH guidelines. Hitherto, there is no article for the quantification and determination of related compounds of molindone in drug substances. This is a novel and sensitive method for the associated compounds in molindone using UPLC.

2. Experimental

2.1. Materials

Clearsynth Labs Ltd., Mumbai, India, supplied molindone and its impurity standards. HPLC grade acetonitrile was purchased from Merck, Darmstadt, Germany. Millipore water was used prepared from the Milli-Q water purification system. The UPLC system was an Agilent 1290 quaternary pump, an autosampler, and a photodiode array detector. The output signal was monitored and processed by Chemstation software from Agilent Technologies, USA [9] on an Intel Core i5 computer (Dell.), water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for hydrolytic studies. Stability studies were carried out in a humidity chamber (Thermo Lab. humidity chamber, India), and photostability studies were carried out in a photostability chamber (Sanyo photostability chamber, Leicestershire, UK).

https://dx.doi.org/10.5155/eurjchem.13.2.180-185.2252

Table 1. Comparison of techniques, detection limits, and the matrix studied.

Techniques	Detection limits	Matrix	References
HPLC	Low detection limit as 50 ng/mL with respect to molindone concentration	Serum samples	[4]
HPLC-MS	25 µM of molindone	Metabolites	[5]
HPLC-MS/MS	0.1-100 ng/mL	Human plasma samples	[6]
HPLC	None	Analyte	[7]
UPLC	0.045 µg/mL (0.009%) for all related compounds with respect to molindone concentration	Drug substance	Current study

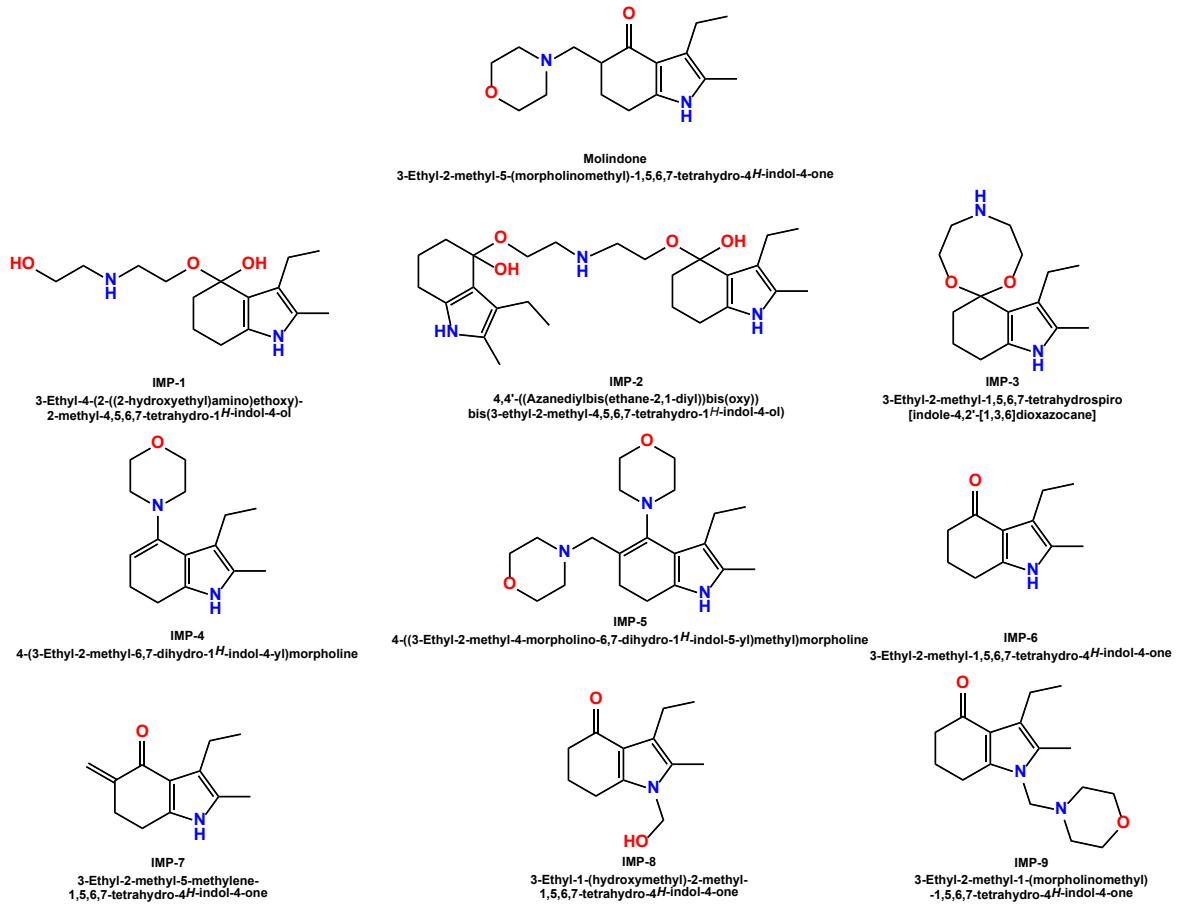

Figure 1. Structures of molindone and its impurities.

Thermal stability studies were performed in a dry air oven (MACK Pharmatech., Hyderabad, India).

2.2. Methods

2.2.1. Chromatographic conditions

InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1×50 mm, 1.9 µm narrow bore LC column manufactured and supplied by Agilent Technologies, USA [9] was used for the separation of molindone and its impurities. The buffer solution was prepared by dissolving 1.1 g of sodium octane sulphonic acid sodium salt in 650 mL of water. Pipetted out 1.0 mL of glacial acetic acid and 0.5 mL of triethylamine, added into the buffer solution. The pH of the buffer solution was adjusted to pH = 5.0 using triethylamine. Mixed 650 mL of buffer solution and 350 mL of methanol as mobile phase. The flow rate of the mobile phase was 0.6 mL/min. The gradient composition of %B at 0.00 min, 5.0%, 0.80 min, 5.0% 2.00 min, 25.0%, 2.40 min, 35.0%, 6.00 min 50.0%, 6.40 min 65.0%, 8.00 min, 80.0%, 9.00 min, 90.0%, 10.00 min, 90.0%, 10.20 min, 5.0%, and 12.00 min, 5.0%. The column oven temperature was maintained at 40 °C, and the detection was monitored at a wavelength of 254 nm. The injection volume was 2.0 µL. Mixed 0.01 N hydrochloric acid and methanol in the ratio of 60:40 (v:v) as diluent.

2.2.2. Preparation of stock solutions and system suitability

Stock solutions of molindone standard and sample (500 µg/mL) were prepared by dissolving appropriate amounts for assay analysis. The standard solution was prepared at a 70 µg/mL concentration from the stock solutions and used as system suitability for related substances determinations.

2.2.3. Preparation of sample solution

Some powdered tablets equivalent to 500 µg/mL of molindone were transferred to a 100 mL volumetric flask, 30 mL of diluent were added and kept on a rotary shaker for 10 min to disperse the material thoroughly, then sonicated for 10 min and diluted to 100 mL (500 µg/mL). The resulting solution was centrifuged at 3,000 rpm for 25 min (the supernatant solution was used for purity evaluation).

2.2.4. Analytical method development

All the related compounds and molindone (Figure 1) had UV maxima at around 254 nm; detection at 254 nm was selected for the method development purpose. To develop a selective and sensitive method, the primary concern during development was to resolve all impurities and its peak symmetry for IMP-1,

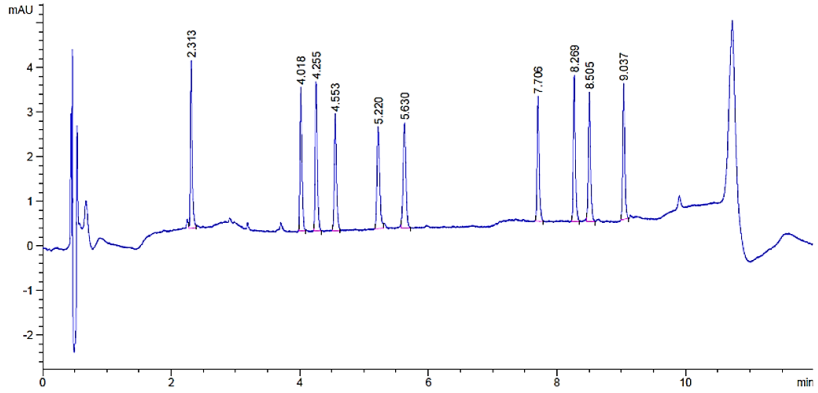

Figure 2. Chromatogram of a representative lot of molindone drug substance. Compound-Retention time (min): IMP-1: 4.0, IMP-2: 2.3, IMP-3: 4.3, IMP-4: 4.6, IMP-5: 5.2, IMP-6: 9.0, IMP-7: 8.5, IMP-8: 8.3, IMP-9: 5.6, and molindone: 7.7.

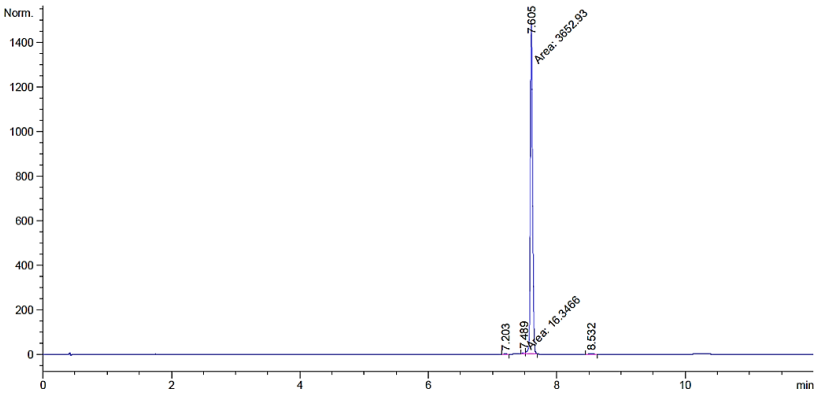

Figure 3. Chromatogram of molindone (7.6 min) and its impurity standards solutions.

IMP-2, IMP-3, IMP-4, IMP-5, IMP-6, IMP-7, IMP-8, IMP-9, and molindone (Figure 1). When the pH moved from basic to acidic, no improvement in symmetry of the molindone peak was observed. At buffer pH = 5.0, the tailing of the molindone peak was 1.4, resolution between molindone and IMP-3 was less (*Rs* < 1), which may be due to the ionization of the amine group in both molindone and IMP-3. Upon study at different pHs, improvement in resolution (*Rs* > 1.8) between IMP-1, molindone, IMP-2, and IMP-3 was observed, and symmetry of the molindone (tailing < 2) peak was obtained. Various ion pairing agents such as trifluoroacetic acid and triethylamine were tested to improve peak properties such as peak height, peak symmetry. The addition of glacial acetic acid and triethylamine to the buffer at pH = 5.0 improved the symmetry of molindone (Tailing factor was enhanced to 1.5).

To improve the symmetry of the molindone peak column, the oven temperature was increased to 40 °C. The symmetry of the molindone peak was improved to 1.2. The effect of various organic modifiers such as acetonitrile, methanol, and different gradient programs was tried to optimize the retention time of molindone and resolution between the impurities. Satisfactory results (the retention time of molindone was ~4.5 min, and the resolution between all impurities was >2.0) were obtained with the above-mentioned chromatographic conditions. Buffer pH and % acetonitrile did not play a major role in separating related compounds and molindone. In the optimized conditions, IMPs 1-9, and molindone were well separated with a resolution greater than 2.0. Analysis was performed for different batches of bulk drug samples (n = 3). Results were within specification. Stability study results as per ICH Q1A (R2) [10] for molindone were generated (Long-term stability 12 months and accelerated stability six months), and the results were well within limits.

2.3. Statistical method

The robustness study was performed using the Minitab 17 software [11] using six factors, viz., flow, column oven temperature, mobile phase organic ratio, the particle size of the packing material, length of the column, and wavelength.

3. Results and discussion

3.1. Analytical method validation

The developed chromatographic method was validated for selectivity, linearity, range, precision, accuracy, sensitivity, robustness, and system suitability.

3.2. Specificity

The specificity of the developed method was assessed by performing forced degradation studies. The specificity of the developed LC method for molindone was determined by related compounds, namely IMPs 1-9. Forced degradation studies were performed on molindone to indicate the stability, indicating properties, and specificity of the proposed method. All stress decomposition studies were performed at an initial drug concentration of 500 µg/mL. The standard and sample chromatograms are shown in Figures 2 and 3.

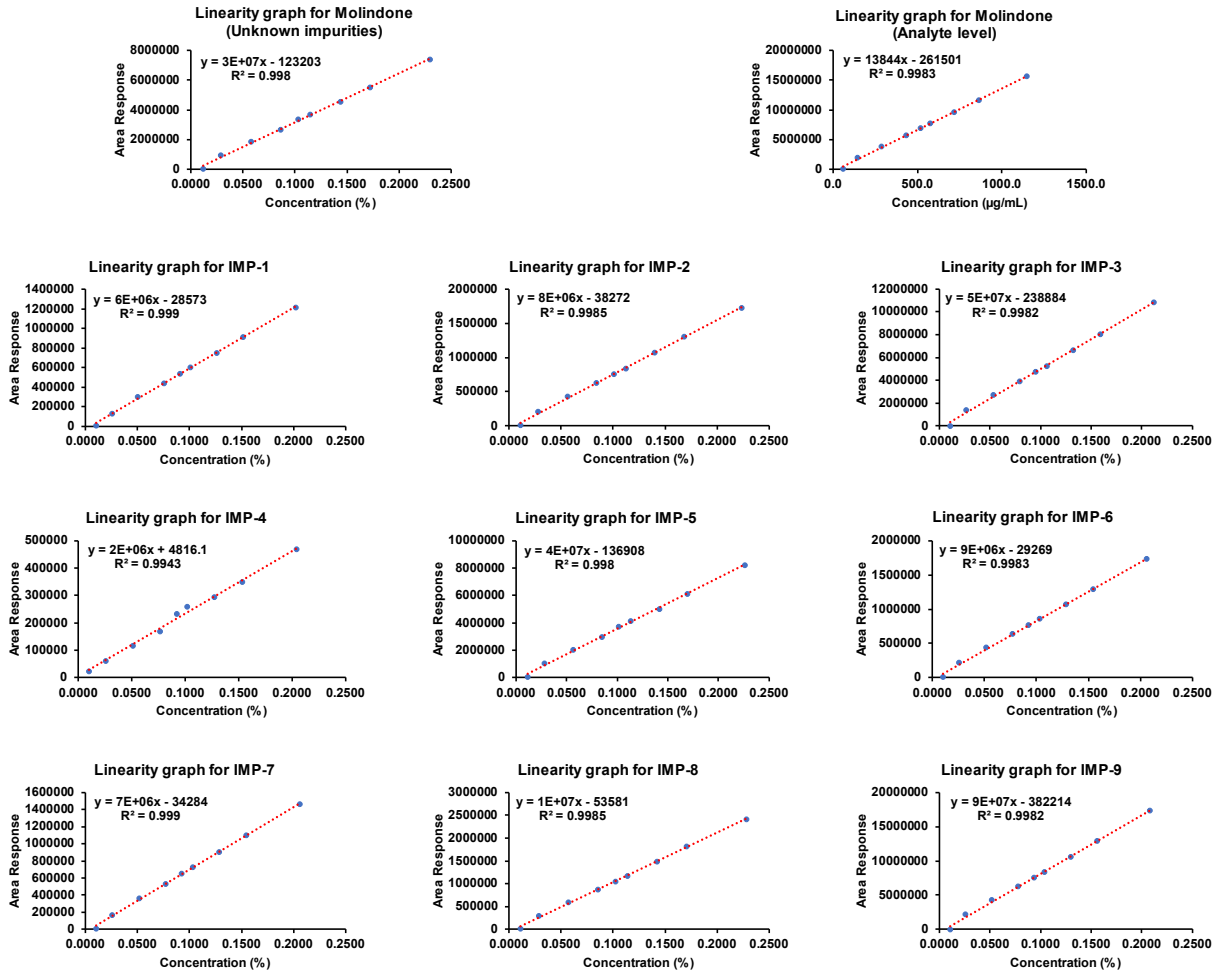

Figure 4. Linearity graph for molindone and IMPs 1-9.

3.3. Results of forced degradation studies

Stress studies on molindone under different stress conditions (carried out as per ICH Q1B [12]), suggested the following degradation behaviour: (i) The drug was exposed to base hydrolysis under reflux conditions. Molindone showed very slight sensitivity towards the treatment of base hydrolysis. Minor degradation was observed when the drug was exposed to 0.1 N NaOH (24 h reflux at 80 °C). (ii) The drug was exposed to acid hydrolysis under reflux conditions. Molindone was not sensitive towards the treatment of acid hydrolysis. No degradation was observed when the drug was exposed to 0.1 N HCl (24 h reflux at 80 °C). (iii) The drug was exposed to 3% hydrogen peroxide at room temperature for 24 h. Molindone showed sensitivity towards the treatment of hydrogen peroxide. With time, the drug gradually degraded in 3% hydrogen peroxide, and significant degradation was observed (~87.7%). (iv) The drug was exposed to water at 60 °C for 24 h. No major degradation products were observed after 24 h. The drug was stable towards water hydrolysis. (v) The drug was stable to the effect of photolysis. When the drug powder was exposed to light for overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200-Watt hours/square meter (W/m.hr) (in a photostability chamber), no degradation was observed. (vi) The drug was stable to the effect of temperature. No degradation was observed when the drug powder was exposed to dry heat at 60 °C for ten days.

Peak purity results for stressed molindone samples, derived from the PDA detector (purity angle within the purity threshold limit), confirm that the molindone and IMPs 1-9 peaks were homogeneous and pure. No degradation product peaks were observed after 30 min in the extended run time of 100 min for all the molindone stressed samples. Assay studies were carried out for stress samples against a qualified reference standard. The mass balance (% assay + % of impurities + % of degradation products) of stressed samples was close to 95.5%, confirming the stability-indicating power of the developed method.

3.4. Linearity and range

The linearity of the method was evaluated by determining nine concentration levels from the limit of quantitation (LOQ) to 200% of 500 µg/mL analyte concentration. The correlation coefficient obtained for molindone was 0.999. The correlation coefficient obtained for IMPs 1-9 and molindone was more significant than 0.999. The linearity graphs IMPs 1-9, and molindone were shown in Figure 4. The linearity of molindone has been studied in two scenarios, *viz*., the first is the concentration from 0.01 to 0.23% level to quantify the unknown impurities in molindone drug substance samples and the latter is the concentrations from 57 to 1150 µg/mL level to quantify the content of molindone (%assay) in drug substance samples.

Table 2. Design of experiments for robustness study.

Experiment	Flow (mL/min)	Column oven temperature (°C)	Wavelength (nm)
1	0.5	35	252
2	0.7	45	256
3	0.7	45	256
4	0.5	35	256
5	0.7	35	256
6	0.7	35	252
7	0.7	45	252
8	0.5	45	252
9	0.5	45	252
10	0.7	35	252
11	0.5	45	256
12	0.5	35	256

3.5. Precision

The precision of the related compounds was checked by injecting six individual preparations of 3.75 µg/mL molindone spiked with 0.15% of each impurity. The % RSD for percentage of IMPs 1-9 and molindone was below 0.4%. The % RSD for IMP was within 0.8% in the intermediate precision. The different analyst columns evaluated the intermediate precision of the method, and by using a different instrument, % RSDs were within 1.8%, confirming the ruggedness of the method.

3.6. Sensitivity

Sensitivity was determined by establishing the limit of detection (LOD) LOD and LOQ for IMPs 1-9, and molindone estimated at a signal-to-noise ratio of 3:1 and 10:1, by injecting a series of dilute solutions with known concentration. The limit of detection for IMPs 1-9 were 0.003%, and for molindone was 0.003% for 2.0 µL injection volume. The limits of quantification for IMPs 1-9 were 0.01% and molindone was 0.01% for 2.0 µL injection volume. The precision study at the LOQ level was performed. The % RSD for the areas of each impurity was within 1.1%. Thus, the method was found to be highly sensitive.

3.7. Accuracy

A recovery study was carried out in triplicate at 50, 100, and 150% of the analyte concentration (500 µg/mL). The percentage of recovery for IMPs 1-9 was 101.3 to 105.1%. The percentage recovery of molindone in bulk drug samples ranged from 99.2 to 101.5%, indicating that the method was suitable for determining related compounds in drug substances.

3.8. Robustness

The robustness study was performed using the Minitab 17 software [11] using three factors viz., flow, column oven temperature, and wavelength. By changing the experimental conditions in Table 2, the resolution between IMPs 1-9, and molindone was evaluated. The flow rate of the mobile phase was 0.6 mL/min. To study the effect of flow rate on the resolution, 0.1 units changed it from 0.5 to 0.7 mL/min. The effect of column temperature on the resolution was studied at 35 and 45 °C instead of 40 °C. The impact of the detection wavelength, 254 nm, transformed it from 252 to 256 nm. In all the deliberate varied chromatographic conditions carried out (flow rate, column oven temperature, and wavelength of detection), the resolution between closely eluting impurities, namely IMPs 1-9, and molindone peaks and molindone, IMP-3 was more significant than 1.5, illustrating the robustness of the method.

3.9. Solution stability

The solution stability of molindone and its related compounds was carried out by leaving the spiked sample solution in a tightly capped volumetric flask at room temperature for 48 hours. The mobile phase stability was performed using freshly prepared sample solutions against reference standard solutions at 48 hours. The %RSD of molindone during solution stability and mobile phase stability experiments was within 1.1%. No significant change was observed in the content of IMPs 1-9, and molindone during solution stability and mobile phase stability experiments. The data confirms that standard and sample solutions were stable for up to 48 hours.

Numerous methods are available [1-4] for the determination of molindone by several techniques like HPLC, UV, LCMS, *etc*. All the plans have determined only the LOD, LOQ, % recovery, and linearity of molindone and not the related compounds of molindone. The data is mentioned in Table 1 for a comparison of different techniques. However, the present method describes the associated compounds of molindone and molindone in both bulk drug substances. All the way describes molindone as the analyte.

4. Conclusion

A new, sensitive, and stability-indicating UPLC method was successfully developed to quantify related compounds of molindone in bulk drugs. The technique was found to be accurate and precise, with excellent and consistent recovery. The validated method may be used for the routine analysis of the determination of related compounds of molindone from the bulk drug, pharmaceutical preparation, and other quality control samples of product development.

Acknowledgements

The authors want to recognize the lab colleagues and research scholars for supporting this research article work.

Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Balaji Nagarajan, Gunasekar Manoharan; Methodology: Gunasekar Manoharan, Nataraj Palaniyappan; Software: Nataraj Palaniyappan; Validation: Ganapathy Narayanan Shanmugam; Formal Analysis: Balaji Nagarajan; Investigation: Balaji Nagarajan; Resources: Ganapathy Narayanan Shanmugam; Data Curation: Abhinav Yarragunta; Writing - Original Draft: Balaji Nagarajan; Writing - Review and Editing: Gunasekar Manoharan; Visualization: Gunasekar Manoharan; Funding acquisition: Gunasekar Manoharan; Supervision: Balaji Nagarajan; Project Administration: Balaji Nagarajan, Gunasekar Manoharan.

ORCID and Email

Balaji Nagarajan
priyabalan8380@gmail.com

balajin@rkpharmainc.com
https://orcid.org/0000-0002-1399-441 7
Gunasekar Manoharan
shekarphd@yahoo.com
https://orcid.org/0000-0001-8553-3874
Ganapathy Narayanan Shanmugam
ganeshnarayan23@gmail.com
https://orcid.org/0000-0001-6649-4486
Nataraj Palaniyappan
palanatraj2020@gmail.com
https://orcid.org/0000-0003-3383-5939
Abhinav Yarragunta
yarraguntaa@gmail.com
https://orcid.org/0000-0002-1443-7532

References

[1]. Junqiu, J.; Meihua, G.; Xiaochang, L.; Jun, C. A kind of synthetic method of improved Molindone. CN:107011237:A, August 4, 2017. https://patents.google.com/patent/CN107011237A/en (accessed April 20, 2022).
[2]. Junqiu, J.; Meihua, G.; Xiaochang, L.; Jun, C. Improved synthesis method of molindone. CN:107011237:B, April 21, 2020. https://patents.google.com/patent/CN107011237B/en (accessed April 20, 2022).
[3]. Hanbauer, M.; Nazir, Z.; Hildebrand, P.; Figini, A.; Liang, L.; Fumagalli, T. Methods of producing molindone and its salts. 9802893, October 31, 2017. https://patents.google.com/patent/US9802893B2/en (accessed April 20, 2022).
[4]. Narasimhachari, N.; Pandurangi, A. K.; Landa, B. A simple HPLC method for the quantitation of molindone in human plasma or serum. *J. Liq. Chromatogr.* **1988**, *11*, 983–989.
[5]. Gawlik, M.; Savic, V.; Jovanovic, M.; Skibiński, R. Mimicking of phase I metabolism reactions of molindone by HLM and photocatalytic methods with the use of UHPLC-MS/MS. *Molecules* **2020**, *25*, 1367.
[6]. Jiang, H.; Li, Y.; Pelzer, M.; Cannon, M. J.; Randlett, C.; Junga, H.; Jiang, X.; Ji, Q. C. Determination of molindone enantiomers in human plasma by high-performance liquid chromatography-tandem mass spectrometry using macrocyclic antibiotic chiral stationary phases. *J. Chromatogr. A* **2008**, *1192*, 230–238.
[7]. Separation of Molindone hydrochloride on Newcrom R1 HPLC column. https://www.sielc.com/separation-of-molindone-hydrochloride-on-newcrom-c18-hplc-column.html (accessed April 20, 2022).
[8]. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ich harmonised tripartite Guideline stability testing: Validation of analytical procedures: text and methodology Q2(R1), https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf (accessed April 20, 2022).
[9]. Chemstation software by Agilent technologies. http://www.chem.agilent.com, https://www.agilent.com/cs/library/usermanuals/Public/G2070-91126_Understanding.pdf (accessed April 20, 2022).
[10]. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ich harmonised tripartite guideline stability testing of new drug substances and products Q1A (R2). https://database.ich.org/sites/default/files/Q1A%28R2%29%20Guideline.pdf (accessed April 20, 2022).
[11]. Minitab Statistical Software, State College, PA: Minitab, Inc. https://www.minitab.com/en-us/products/minitab/ (accessed April 20, 2022).
[12]. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ich harmonised tripartite Guideline stability testing: Photostability testing of new drug substances and products. Q1B https://database.ich.org/sites/default/files/Q1B%20Guideline.pdf (accessed April 20, 2022).