Iranian Journal of Organic Chemistry Vol. 9, No. 3(2017) 2117-2121

A novel method for purification of the synthesized Br-HIDA compound and its identification by FT-IR and HPLC techniques in Pars Isotope Company

Mehdi Nabati*and Hossein Mohammadnejad-Mehrabani

Synthesis and Molecular Simulation Laboratory, Chemistry Department, Pars Isotope Company, P.O. Box: 1437663181, Tehran, Iran

Received: May2017; Revised: June 2017; Accepted: July 2017

Abstract: In the present article, N-[2,4,6-trimethyl-3-bromoacetanilid]iminodiacetic acid (bromo-HIDA, or Br-HIDA) compound was synthesized in three steps. The Technetium-99m-labeled mebrofenin, transported in blood by binding to albumin in the same manner as the indocyanine green (ICG) clearance test, can be used for hepatobiliary scintigraphy in the assessment of liver function. The important goal of this research work was to purify the Br-HIDA molecule by our novel method (wash with acidic solution and then solvent-antisolvent technique). Then, its purification was identified by HPLC and FT-IR analyses and compared with standard obtained bromo-HIDA. The comparison between the HPLC analyses of our purified mebrofenin and obtained compound shows high accuracy of our method.

Keywords: Br-HIDA, FT-IR spectroscopy, HPLC technique, Identification, Purification, Radiopharmaceutical.

Introduction

Nuclear medicine is a branch of medical imaging that uses small amounts of radioactive material to diagnose and determine the severity of or treat a variety of diseases, including many types of cancers, heart disease, gastrointestinal, endocrine, neurological disorders and other abnormalities within the body. Because nuclear medicine procedures are able to pinpoint molecular activity within the body, they offer the potential to identify disease in its earliest stages as well as a patient's immediate response to therapeutic interventions. Nuclear medicine imaging procedures are noninvasive and, with the exception of intravenous injections, are usually painless medical tests that help physicians diagnose and evaluate medical conditions. These imaging scans use radioactive materials called radiopharmaceuticals or radiotracers. Depending on the type of nuclear medicine exam, the radiotracer is either injected into the body, swallowed or inhaled as a gas and eventually accumulates in the organ or area of the body being examined.

*Corresponding author. Tel: +982188337023 Fax: +982188337024, E-mail: mnabati@ymail.com

Radioactive emissions from the radiotracer are detected by a special camera or imaging device that produces pictures and provides molecular information. Nuclear medicine also offers therapeutic procedures, such as radioactive iodine (I-131) therapy that use small amounts of radioactive material to treat cancer and other medical conditions affecting the thyroid gland, as well as treatments for other cancers and medical conditions [1-4].

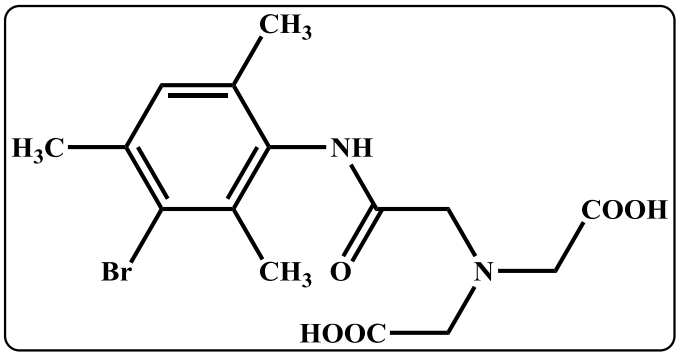
Hepatic resection is the therapy of choice for malignant and symptomatic benign, hepatobiliary tumors. Recent years have shown a marked decrease in morbidity and mortality rates after major liver resections. Refinements in operative techniques, better selection of patients and advances in peri-operative care are thought to be responsible for this improvement. The Technetium-99m-labeled Iminodiacetic acid (IDA) analogues, transported in blood by binding to albumin in the same manner as the indocyanine green (ICG) clearance test, can be used for hepatobiliary scintigraphy in the assessment of liver function. In liver transplant patients, hepatobiliary

scintigraphy has been performed to obtain information about the functional and morphological status of the graft. Hepatobiliary scintigraphy, requiring a single intravenous injection, provides visual and quantitative information of global and regional liver function as well as excretory function (intrahepatic and extrahepatic bile transport). 99mTc-Mebrofenin is excreted in bile by the hepatocytes by the ATP-dependent export pump multidrug-resistance associated protein 2 (MRP 2), without undergoing biotransformation during their transit through the hepatocyte. Therefore, this agent is well suited for the study of hepatic transport [5].

The mebrofenin (bromo-HIDA, or Br-HIDA) compound is synthesized and its kit formulation is performed in Pars Isotope Company. The aim of this study is purification of this API by novel method and its identification by FT-IR and HPLC spectroscopy techniques.

Results and discussion

The molecular structure of N-[2,4,6-trimethyl-3-bromoacetanilid]iminodiacetic acid (bromo-HIDA, or Br-HIDA) is shown in Scheme **1**.



Scheme 1: The molecular structureof Br-HIDA.

Conclusions

In summary, in this present research work we have synthesized the Br-HIDA compound from 2,4,6-trimethyl aniline in three steps. The main of this work was to purify the final compound with novel method (wash with acidic solution and then solvent-antisolvent technique). Then, its purification was identified by HPLC and FT-IR analyses and compared with standard obtained mebrofenin. The comparison between the HPLC analyses of our purified mebrofenin and obtained compound shows high accuracy of our method.

Experimental

Materials:

The 2,4,6-trimethyl aniline, ethanol, chloroacetyl chloride, acetic acid, HCl 37%, NaOH, iminodiacetic acid, bromine and sodium acetate were obtained from Merck Company. All the solvents were distilled and stored over a drying agent.

***Measurements*:**

Infrared spectra were recorded with a 4600 Unicam FT-IR spectrophotometer as KBr pellets. The compound purification was identified by HPLC apparatus. The chromatographic apparatus consisted of a Jasco (Tokyo, Japan) PU-1580 isocratic pump and a Jasco UV-1575 spectrophotometric detector, a Rheodyne 7725i manual injector equipped with a 20 µL loop (Rheodyne, Cotati, CA, USA). The chromatographic system was controlled by HSS-2000 provided by Jasco using the LC-Net II/ADC interface.

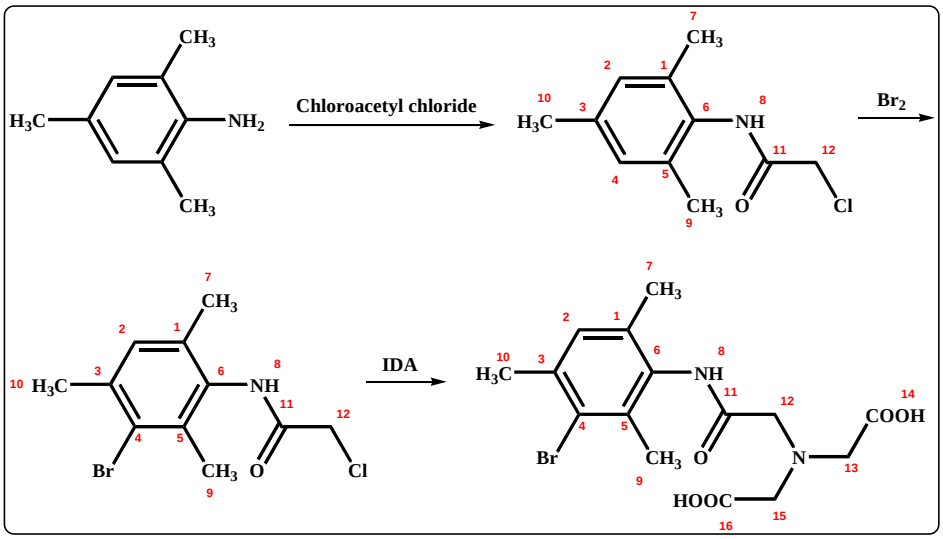
Synthesis and purification processes:

The synthesis of this compound is done in three steps in our laboratory (Scheme **2**).

Firstly, 2,4,6-trimethyl aniline (17.7 mL) is dissolved in glacial acetic acid (78 mL) and cooled to 5 ºC in an ice bath. To this, 9.9 mL of chloroacetyl chloride in 45 mL of glacial acetic acid is added in a drop wise manner with continuous stirring, using a funnel with a stopcock. The temperature of the reaction mixture is maintained at less than 10 ºC during the addition of the chloroacetyl chloride. To this is added 31.5 g of sodium acetate dissolved in 134 mL of water. The reaction mixture is stirred for an additional 30 min.

The product (white solid) is filtered, washed eight times with water and dried at 70 ºC temperature. The obtained product weight is 23 g.

In second step, the previous synthesized compound (14.45 g) is dissolved in glacial acetic acid (100 mL) and heated under reflux in an oil bath. Then, bromine (3.25 mL) in glacial acetic acid (29 mL) is added in a



Scheme 2. The synthesis process of Br-HIDA compound.

drop wise manner and cooled to 45 ºC temperature. This reaction is performed for 16 h at 45 ºC temperature. After this time, 0.54 g sodium metabisulfite in 204 mL distilled water is added in a drop wise manner with continuous stirring, using a funnel with a stopcock, to the reaction mixture. The composed white solid product is filtered, washed six times with water and two times with diethyl ether. The compound is dried at 70 ºC temperature. The obtained product weight is 33 g.

Finally, *N*-(3-bromo-2,4,6-trimethylphenyl)-2-chloroacetamide (20.39 g), prepared as described above, is dissolved in 128 mL of 95% ethanol and heated under reflux. To this, 9.17 g of iminodiacetic acid and 12.45 g sodium hydroxide, dissolved in 128 mL of distilled water, is added using a funnel. The reaction mixture is heated and stirred until total dissolution occurs. Refluxing is continued for 18 h at 60 ºC temperature. The ethanol is evaporated at 79–80ºC by rotary apparatus. The pH of the solution is adjusted to 2-3 using 5 N HCl; the solution is kept refrigerated overnight. The resulting white precipitate is filtered and washed three times with 0.01 N HCl. Then, the precipitate is solved in hot 60% ethanol and the activated coal is added to this solution. The solution is filtered and kept refrigerated overnight. The resulting white precipitate is gathered and washed three times with diethyl ether. The compound is dried at 70 ºC temperature. The obtained product weight is 15 g.

2-Chloro-N-mesitylacetamide:

White powder, yield: 95% and melting point: 112°C. Mass analysis (m/z): 211.8 (100%), 212.08 (12.1%), 213.07 (32%) and 214.08 (3.9%). Elemental analysis: C, 62.41%; H, 6.67% and N, 6.62%. IR (neat, cm^{-1}): 3236, 3038, 1671, 1608, 1540, 1485, 1333, 1241, 1202, and 1039. ^{1}HNMR, δ: 2.20 ($6H_{7,9}$ and singlet), 2.26 ($3H_{10}$ and singlet), 4.32 ($2H_{12}$ and singlet), 6.90 ($2H_{2,4}$ and singlet) and 10.02 ($1H_{8}$ and singlet). ^{13}CNMR, δ: 17.9 ($C_{7,9}$), 21.9 (C_{10}), 42.7 (C_{12}), 128.1 ($C_{2,4}$), 134.1 ($C_{1,5,6}$), 136.4 (C_{3}) and 165.4 (C_{11}).

N-(3-bromo-2,4,6-trimethylphenyl)-2-chloroacetamide:

White powder, yield: 95% and melting point: 118°C. Mass analysis (m/z): 290.98 (100%), 288.99 (77.4%), 292.98 (24.1%), 291.99 (12.1%), 289.99 (9.4%) and 293.99 (3.0%). Elemental analysis: C, 45.47%; H, 4.51% and N, 4.82%. IR (neat, cm^{-1}): 3243, 3100, 3023, 1662, 1581, 1527, 1457, 1419, 1384, 1332, 1234, 1164, and 1030. ^{1}HNMR, δ: 2.08 ($3H_{7}$ and singlet), 2.25 ($3H_{9}$ and singlet), 2.33 ($3H_{10}$ and singlet), 4.32 ($2H_{12}$ and singlet), 6.96 ($1H_{2}$ and singlet) and 10.02 ($1H_{8}$ and singlet). ^{13}CNMR, δ: 17.9 (C_{7}), 18.1 (C_{9}), 24.4 (C_{10}), 42.7 (C_{12}), 124.5 (C_{4}), 129.4 (C_{3}),

130.3 (C_2), 132.7 (C_5), 133.1 (C_1), 136.3 (C_6) and 165.4 (C_{11}).

2,2'-((2-((3-bromo-2,4,6-trimethylphenyl)amino)-2-oxoethyl)azanediyl)diacetic acid:

White powder, yield: 78% and melting point: 198˚C. Mass analysis (m/z): 386.05 (100%), 388.05 (99.7%), 387.05 (16.6%), 389.05 (16.2%) and 390.05 (2.3%). Elemental analysis: C, 46.53%; H, 4.95% and N, 7.23%. IR (neat, cm^{-1}): 3447, 3273, 3012, 2964, 1671, 1541, 1378, 1341, and 1262. ^{1}HNMR, δ: 2.08 ($3H_7$ and singlet), 2.25 ($3H_9$ and singlet), 2.33 ($3H_{10}$ and singlet), 3.30 ($4H_{13,15}$ and singlet), 3.34 ($2H_{12}$ and singlet), 6.96 ($1H_2$ and singlet), 9.08 ($1H_8$ and singlet), and 13.03 ($2H_{COOH}$ and singlet). ^{13}CNMR, δ: 17.9 (C_7), 18.1 (C_9), 24.4 (C_{10}), 58.5 (C_{12}), 59.5 (C_{COOH}), 124.5 (C_4), 129.4 (C_3), 130.3 (C_2), 132.7 (C_5), 133.1 (C_1), 136.3 (C_6) and 168.5 (C_{11}).

Also, Purified mebrofenin was compared with the standard by HPLC apparatus (Figures 1-3). Compound was injected in a 250 mm × 4.6 mm ID, 5-µm particle, Perfectsil Target ODS-3 column (MZ-Analysentechnik, Germany) with a ODS-3 precolumn (10×4.0 mm I.D., 5-µm), which was maintained at ambient temperature. The isocratic mobile phase consisted of methanol, and the flow rate was 1 $mL.min^{-1}$. The mobile phase was filtered, before using, through a 0.45-µm Millipore filter and degassed ultrasonically. The comparison between the HPLC analysis of the purified mebrofenin and obtained Br-HIDA shows that our method is a good technique to purification of this compound.

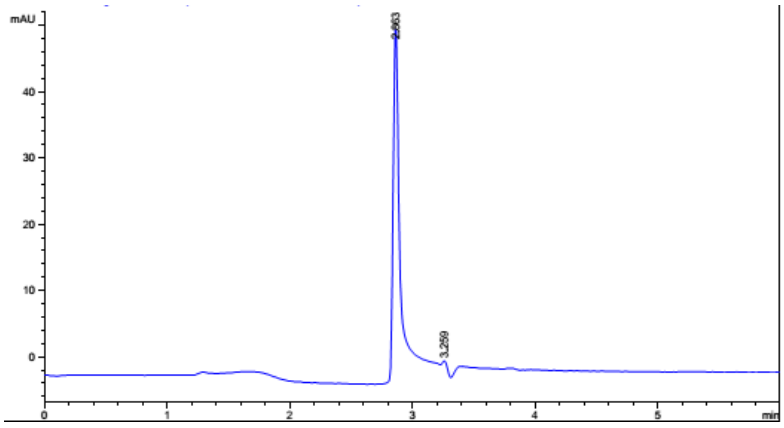


Figure 1: HPLC analysis graph of purified mebrofenin.

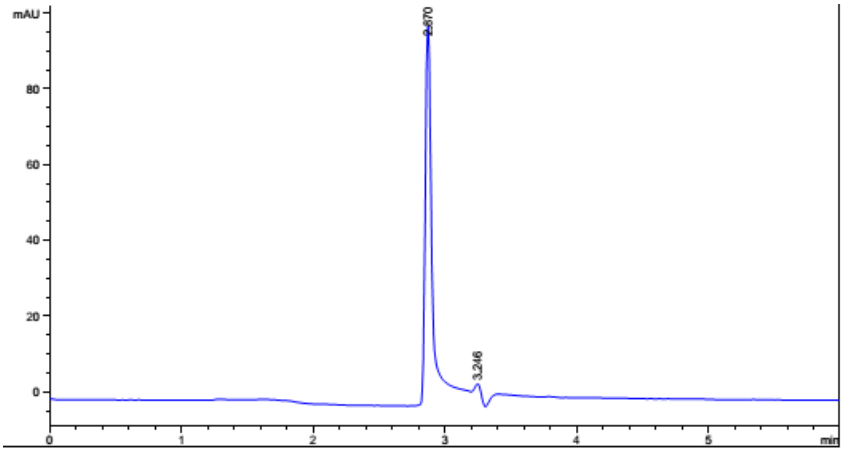


Figure 2: HPLC analysis graph of obtained mebrofenin from ABX Company.

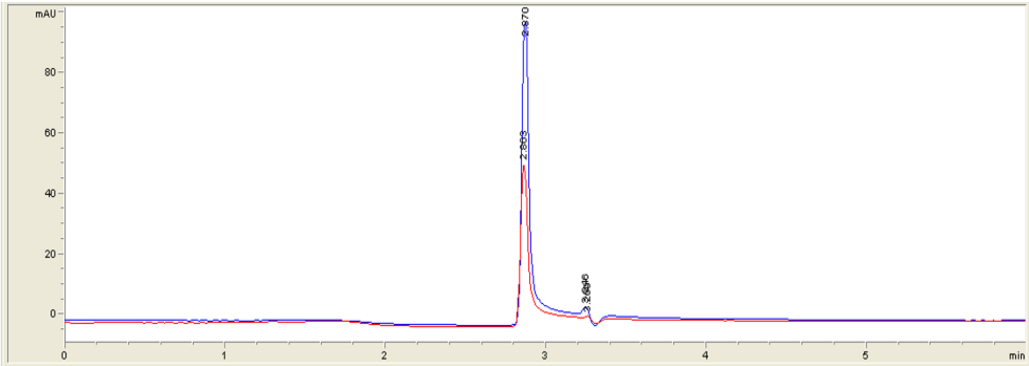


Figure 3: The compared HPLC analyses of purified Br-HIDA (blue line) and obtained compound (red line).

Acknowledgments

The corresponding author is grateful to Doctor Hojjatollah Salehi and Mr. Hossein Abbasi for providing valuable suggestions.

References

[1] Fallahi, B.; Esmaeili, A.; Beiki, D.; Oveisgharan, S.; Noorollahi-Moghaddam, H.; Erfani, M.; Tafakhori, A.; Rohani, M.; Fard-Esfahani, A.; Emami-Ardekani, A.; Geramifar, P.; Eftekhari, M. *Ann. Nucl. Med.*, **2016**, *30*, 153-162.
[2] Erfani, M.; TShafiei, M. *Nucl. Med. Biol.*, **2014**, *30*, 317-321.
[3] Erfani, M.; TShafiei, M.; Charkhlooie, G.; Goudarzi, M. *Iran J. Nucl. Med.*, **2015**, *23*, 15-20.
[4] Nabati, M.; Salehi, H. *Iran. J. Org. Chem.*, **2017**, *9*, 2013-2023.
[5] Liu, S. *Chem. Soc. Rev.*, **2004**, *33*, 445-461.