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A novel two-step method for synthesis, purification and characterization of mebrofenin in Pars Isotope Company

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Abstract: This article presents the novel method for synthesis of the N-[2,4,6-trimethyl-3-bromoacetanilid]iminodiacetic acid (bromo-HIDA, or Br-HIDA) compound in two 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 synthesize and purify the mebrofenin molecule by our novel method. Then, its purification was identified by NMR, HPLC and FT-IR analyses and compared with standard obtained bromo-HIDA. The comparison between the HPLC analyses of our synthesized mebrofenin and obtained compound shows high accuracy of our method.

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

Introduction

Radiopharmaceuticals are radioactive agents, which may be used to find and treat certain diseases or to study the function of the body's organs. A radiopharmaceutical 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.

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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. 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]. Technetium (^{99m}Tc) mebrofenin (BrHIDA) is a diagnostic radiopharmaceutical with no known pharmacologic action at recommended imaging doses. ^{99m}Tc-

mebrofenin is indicated for imaging of the liver and the gallbladder. Upon intravenous administration, ^{99m}Tc-mebrofenin bound to plasma proteins is cleared from systemic circulation in approximately 5 minutes by hepatocytes, while maximal liver uptake occurs within 11 minutes. Mechanism of mebrofenin entering the gallbladder is thought to occur with a mechanism similar to bilirubin clearance. Normal adult patient (70 kg) dose with normal serum bilirubin levels of less than 1.5 mg/dL is 2 to 5 mCi. Increased serum bilirubin increases renal clearance, decreases hepatic uptake and increases visualization time, thus a higher dose of 3 to 10 mCi is recommended. Doses higher than 10 mCi are seldom used. Obese patients require increased dose of 2 to 3 mCi compared with the normal adult patient dose to obtain proper visualization [5].

Mebrofenin is a chelate composed of two molecules of a lidocaine analogue, attached to a technetium-99m ion. All of the hepatobiliary visualization agents previous to mebrofenin have the same structural composition with changes only of the substituants on the phenyl ring of the lidocaine analogue molecules. Mebrofenin's fast hepatic excretion ($t_{½}$=17 minutes) and high hepatic uptake (98.1%) can be attributed to the 3-bromo-2,4,6-trimethylphenyl moiety [6]. To be a good hepatobiliary imaging agent, the chemical structure of mebrofenin has to meet certain requirements:

- An organic ion with molecular weight between 300 and 1000
- At least two aromatic rings in the molecule
- Ability to bind to albumin

In normal individuals, uptake of ^{99m}Tc-mebrofenin by hepatocytes is 100%. Decreased liver uptake is indicative of hepatocyte disease. Once in the hepatocytes, ^{99m}Tc-mebrofenin is secreted into the canaliculi and finally excreted by the bile ducts [6].

The mebrofenin (bromo-HIDA, or Br-HIDA) compound is synthesized with high purity and its kit formulation is performed in Pars Isotope Company. Recently, we have succeeded to synthesis the mebrofen in with purity 100 percent by Young-Don Hong [7] method. In fact, we optimize the mentioned synthesis method. The aim of this study is synthesis and purification of this API by novel method and its identification by FT-IR, NMR 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**.

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

White powder, yield: 9.3% and decomposing point: 176-178 ˚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 synthesized mebrofenin and obtained mebrofenin shows that our method is a good route to production of this compound.

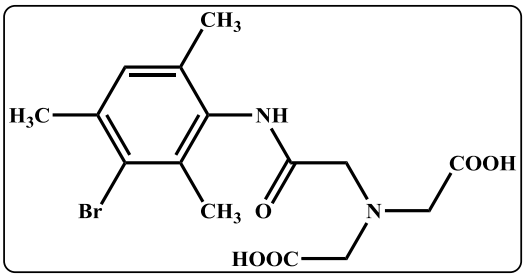
Synthesis and purification processes

The synthesis of this compound is done in two steps but one step purification in our laboratory (Scheme **2**).

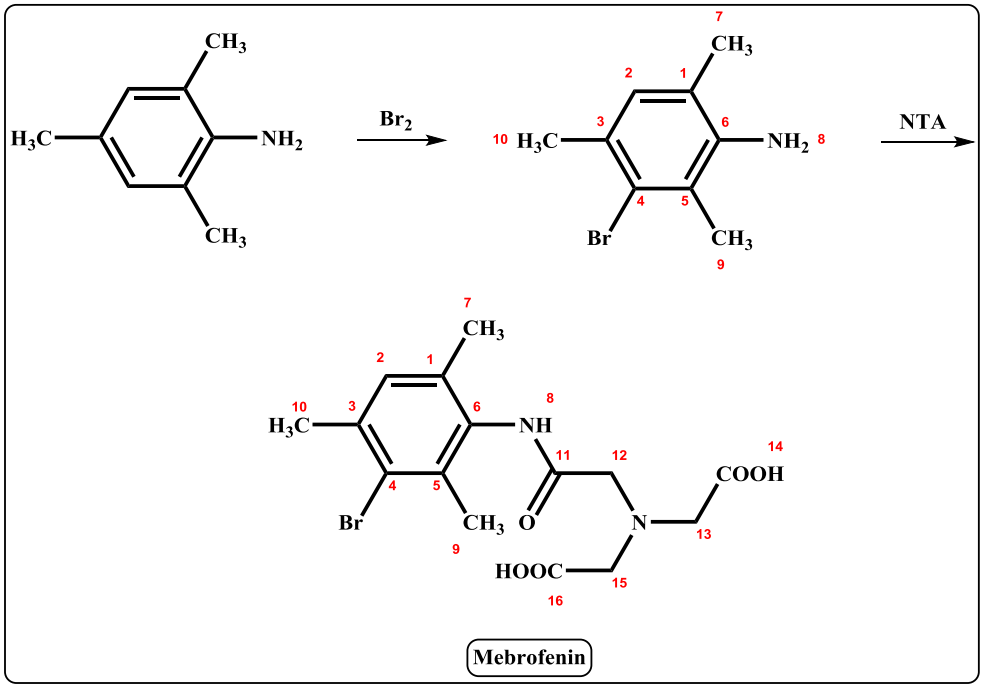
Firstly, To 120 ml of HCl 37% continually stirred in an ice bath 12 ml of 2,4,6-trimethylaniline was added dropwise to obtain a yellowish suspension of 2,4,6-trimethylaniline hydrochloride salt. Then, 4.5 ml of bromine diluted in 80 ml of concentrated HCl 37% was added dropwise to the suspension. After the addition of bromine was complete, the mixture was stirred gently at 44 ˚C for 4 hours to avoid frothing. The resulting solid was poured into 800 ml distilled water. Then, the solution was filtered, washed with 1 N HCl solution, and dried under a vacuum. It was then dissolved in hot water, followed by an addition of liquid ammonia 32% to adjust the pH to 10, and then extracted with diethyl ether. The organic layer was collected, dried with $MgSO_4$, and evaporated to yield brown oil liquid. This brown liquid was used for the next reaction without any further purification.

In second step, 5 g of Nitrilotriacetic acid (NTA) was suspended in 120 ml of anhydrous pyridine. Pyridine

has two important roles in this reaction as a solvent and basic property. The mixture was heated at 110˚C under a nitrogen atmosphere until the nitrilotriacetic acid (NTA) was completely dissolved. 8 ml of acetic anhydride was rapidly introduced to the stirred solution, which was then refluxed at 110˚C for 4 hours and left at room temperature overnight. After that, the solution was heated to 70 ˚C and 3-bromo-2,4,6-trimethylaniline was dissolved in 80 ml pyridine and added to the reaction mixture, and heated at 110˚C for 4 hours and left at room temperature overnight. The solvent was evaporated off in a rotary evaporator. The brown oil residue was dissolved in methanol and treated with activated charcoal to decolorize the mixture; after which, the solvent was evaporated. Double distilled water was added to the mixture, and the solution was adjusted to pH = 10 with a 1 N NaOH solution followed by an extraction with diethyl ether. The aqueous layer was acidified to a pH of 2 with 2 N HCl. The precipitate was filtered and recrystallized from 85% ethanol to yield 3.2 g of the desired product.



Scheme 1: The molecular structure of mebrofenin.



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

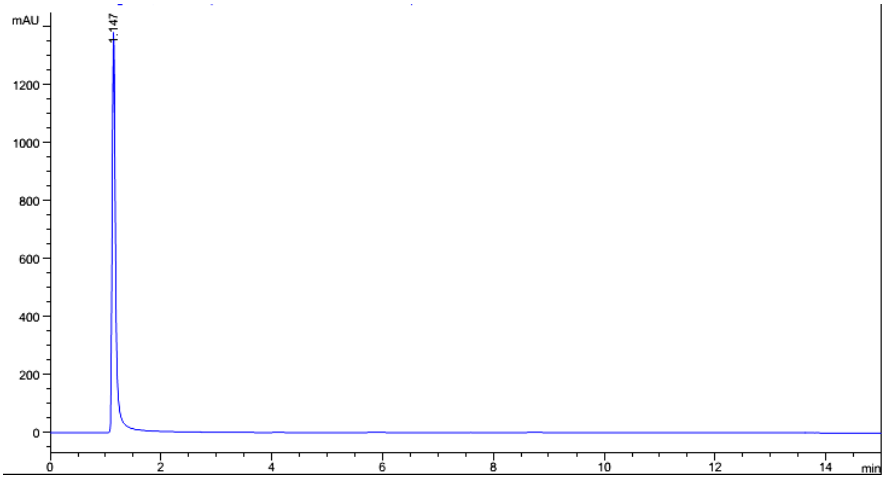


Figure 1: HPLC analysis graph of the synthesized mebrofenin.

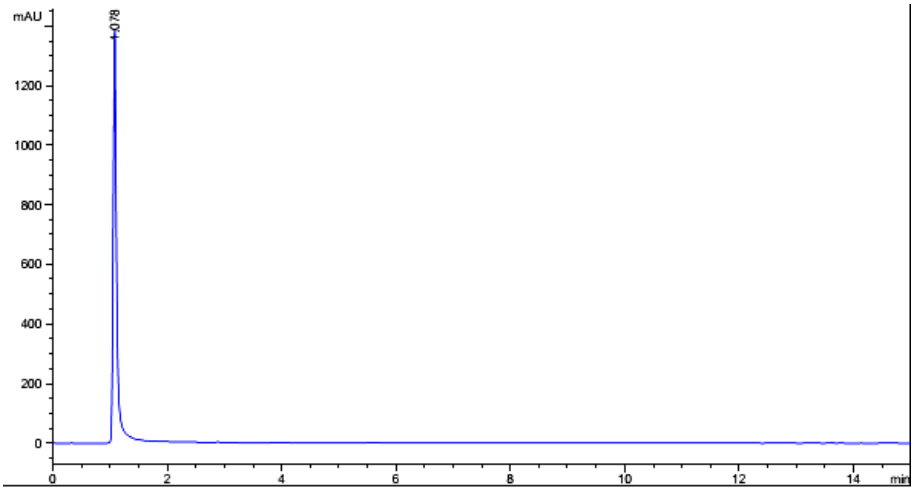


Figure 2: HPLC analysis graph of obtained mebrofenin from ABX Company.

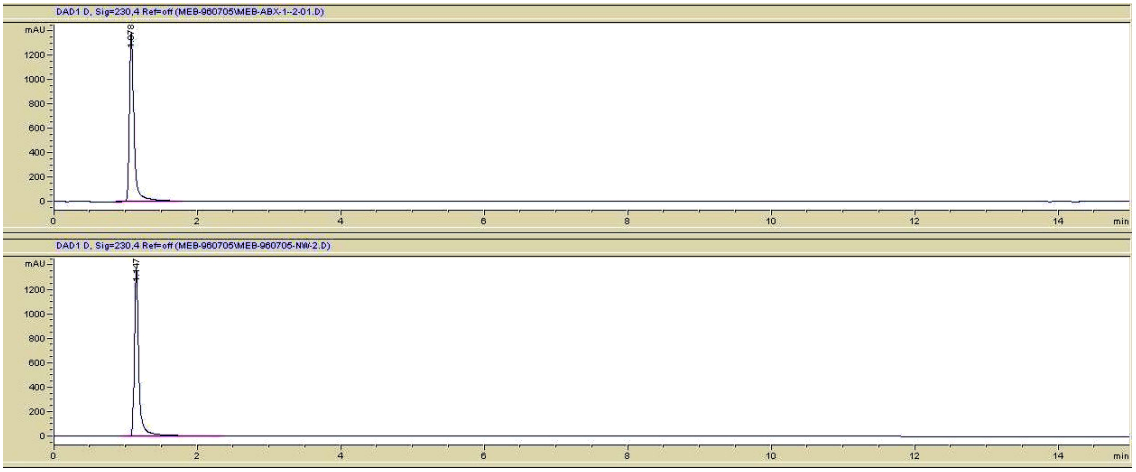


Figure 3: The compared HPLC analyses of the synthesized mebrofenin (low graph) and obtained compound (top graph).

Experimental Section

Materials:

The 2,4,6-trimethyl aniline, ethanol, pyridine, methanol, HCl 37%, NaOH, acetic anhydride, nitrilotriacetic acid (NTA), bromine and diethylether (DEE) 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.

Conclusions

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

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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