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ALKYLATING AGENT BENDAMUSTINE HYDROCHLORIDE FOR TREATMENT OF CANCER DISEASE

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ABSTRACT

Nowadays exposure level in the environment, which is really a concern to increase number of cancer patients in the society. Therefore, it is duty for all human being to take necessary action against the deadly disease by controlling cancer generating chemicals and its proper disposal and storage. Bendamustine is an important anticancer drug for the treatment of CLL and other life threatening disease. The main focus of this review is to discuss about the developed analytical methods, and impurity due to degradation and hydrolysis. All methods are validated according to ICH guidelines and regulatory aspects. The methods are easy, required derivatization and sometime no need of that, directly can be analyze in its pure form, formulation and biological fluids. The impurities and degradation products were reported and confirmed structure presented with suitable literature. This review can able to enhance interest for new researcher in the field of pharmaceuticals with respect to anticancer drugs.

KEYWORDS: Drugs, Bendamustine, Impurity, UV–Visible, HPLC, LCMS.

INTRODUCTION

Bendamustine hydrochloride (Figure 1), a nitrogen active mustard agent, well-known 1H-benzimidazole-2-butanoic acid, 5- [bis (2-chloroethyl) amino]-1 methyl mono hydrochloride.[1] It is mainly useful for the treatment of chronic lymphocytic leukemia (CLL), breast cancer, myeloma as well for hodgkin disease. First synthesized in 1963 and was introduced in the market in Germany with name Cytostasan in the year 1971.[2–6] But approved by USFDA in the year 2008 for non-hodgkin lymphoma (NHL) and CLL.

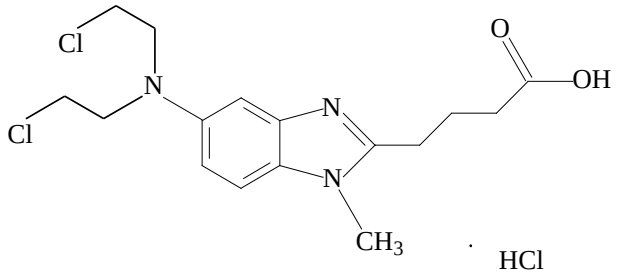

Figure 1: Structure of Bendamustine hydrochloride.

Its formulation is available in the market in the form of injection with brand name Treanda.[7] It decreases the estrogen hormone, which is responsible to enhance the breast cancer cell for female, by degrading and leave the binder receptor of the hormone. Therefore, this drug has the ability to reduce estrogen and inhibits growth of cancer cell. It involved mechlorethamine, benzimidazole and butyric acid as a substituent in its moiety. As a result, it formed electrophilic alkyl group, consequently responsible to make covalent bond with available nucleophilic substituent having electron rich moieties. This drug was administered in the body in lyophilized form because it reconstitutes again in the presence of water. Due to hydrolysis, two possible products are expected and detected, also known as biotransformation product (Figure 2), 4-f5-[bis-(2-hydroxyethyl)amino]-1-methyl-1H benzimidazol-2-ylg butanoic acid (1a) and monohydroxy and dihydroxy derivatives (4-f5-[(2-chloroethyl)-(2-hydroxyethyl) amino]-1-methyl-1Hbenzimidazol-2-ylg butanoic acid (1b).[8]

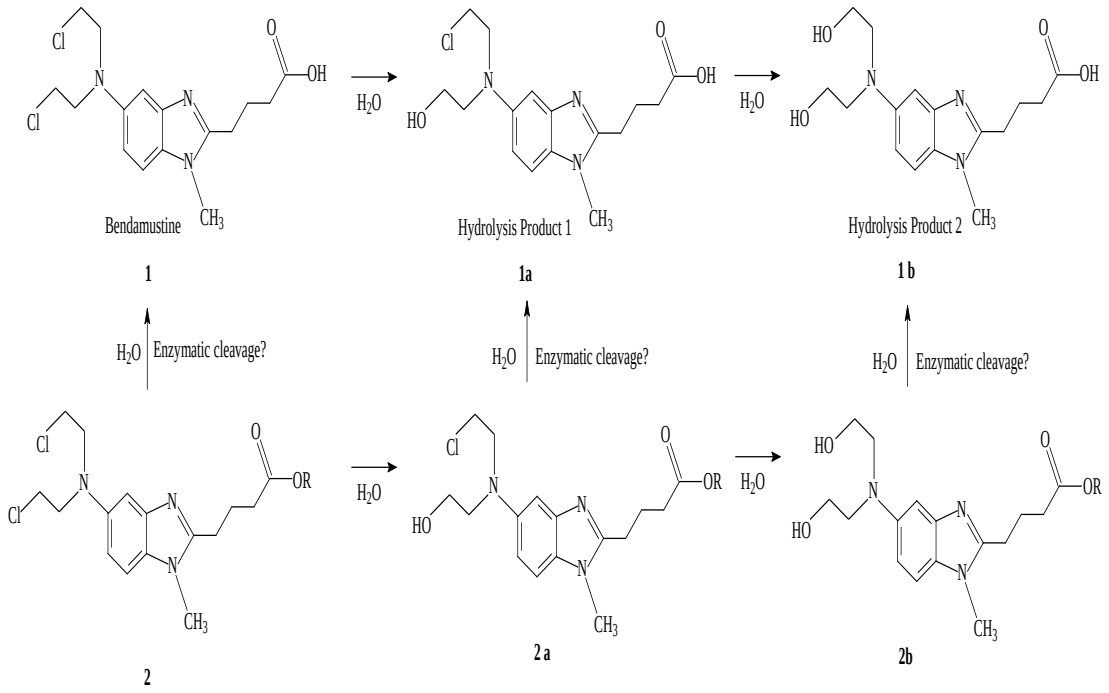

Figure 2: Possible degradation product of bendamustine due to hydrolysis.

IMPURITY

Impurities present in the active pharmaceutical ingredients (API) are classified into three categories, mainly residual solvents, inorganic and organic impurities based on regulatory requirements as per International Conference on Harmonization (ICH) guidelines.[9] It is necessary to identify and characterize the available impurity to assure about safety and quality of the drugs and its pharmaceutical products. Organic impurities may arise from starting materials, synthetic intermediates and degradation product. It depends on reaction conditions such as temperature, pH or in storage condition. Inorganic impurities present in pharmaceutical products originate from the equipment used and from reagents, catalysts, filter aids and drying agents. Residual solvents and other volatile impurities not only detected, need to calculate assayed, because of their potential toxicity, and harsh environmental effects. It has five possible impurities (Figure 3a, 3b, 3c, 3d, 3e), generated due to hydrolysis, oxidation, photolysis and thermal degradation.[10]

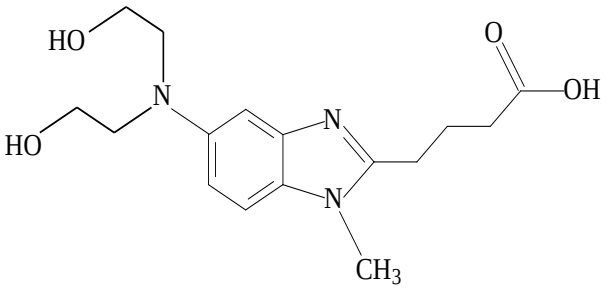

Figure 3a: HP-2: 4-f5-[bis-(2-hydroxy-ethyl)-amino]-1-methyl-1H-benzoimidazol-2-ylg-butyric acid.

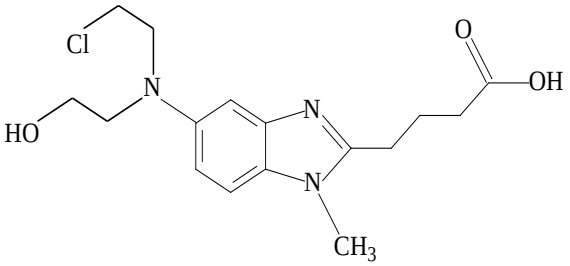

Figure 3b: HP-1: 4-f5-[(2-chloro-ethyl) -(2-hydroxy-ethyl)- amino]-1-methyl-1H-ben zoimidazol-2-ylg-butyric acid.

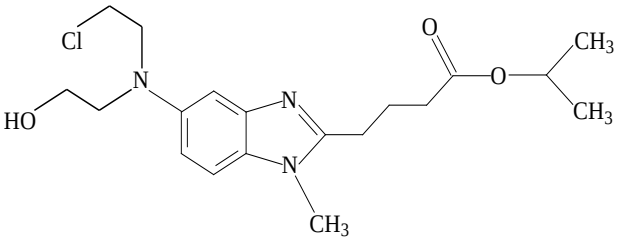

Figure 3c: HP-1 ester: 4-f5-[(2-chloro-ethyl)-(2-hydroxy-ethyl)-amino]-1-methyl-1H benzoimidazol-2-ylgbutyric acid isopropyl ester.

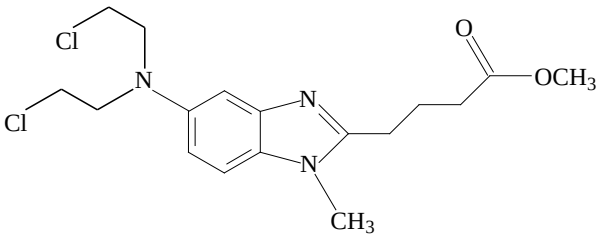

Figure 3d: Methyl ester: 4-f5-[bis-(2-chloroethyl)-amino]-1-methyl-1H-benzoimidazol- 2-ylg-butyric acid methyl ester.

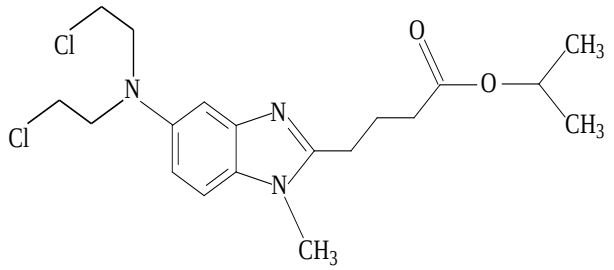

Figure 3e: BEN-2: 4-f5-[bis-(2-chloro-ethyl)-amino]-1-methyl-1H-benzoimidazol-2-ylg-butyric acid isopropyl ester.

ANALYTICAL TECHNIQUES

UV–Visible Spectrophotometer

The molecule absorb light in the ultraviolet and visible region, normally from 200–800 nm based on their ability. Generally, Beer–Lambert law will be followed to determine the concentration by varying wavelength. At the end, one wavelength at which absorbance was maximum can be selected, and then correlation is developed between concentrations with absorbance. Two radiation sources, deuterium and tungsten halogen lamp were used for wavelength below 320 nm and above respectively. The demonstrations are simple as well as sensitive, fast and economical for medicinal drugs and its dosage forms. The advantages of these methods are low time and labor consumption. The accuracy, precision of these methods is excellent. The visible spectrophotometric method was based on complex-formation reaction, oxidation–reduction in other word redox reaction. Three methods were developed within ultraviolet range for quantitation of bendamustine in pharmaceutical dosage form.[11] All methods were validated according to ICH guidelines. Colored complexes were formed and quantified using tropaeolineo-oo, alizarin red S, 1:10 phenanthroline, alkaline potassium permanganate in bulk and pharmaceutical formulations.[12–14] The detailed survey about bendamustine with respect to uv-visible spectrophotometer is tabulated in Table 1.

Table 1: Determination of bendamustine using UV–Visible spectrophotometer.

Reagent/Solvent	λ_{max} (nm)	Linear Range (µg/ml)	Application	Reference
Phosphate buffer, pH=6.8	232.41	0.1–50	Pharmaceutical formulations	[11]
Boric buffer, pH=9	229.25	0.5–50	Pharmaceutical formulations	[11]
Tropaeolineo-oo	480	2.5–12.5	Bulk and pharmaceutical formulations	[12]
Alizarin Red S	460	2.5–12.5	Bulk and pharmaceutical formulations	[12]
$FeCl_3$/1,10-phenanthroline	510	5–40	Bulk drug and formulation	[13]
$KMnO_4$	610	8.5–51	Pure drug and pharmaceuticals	[14]

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) was previously known as high pressure liquid chromatography. The modern instrumentation can control the pressure of the stationary phase renamed with performance. This instrument is really an important device for pharmaceutical analysis to quantify and calculate assayed of the available impurity, generated during synthesis or developed after degradation. Accurate, simple and reproducible HPLC methods were available in the literature for investigation of bendamustine in bulk, pharmaceutical formulations and biological fluids. Different combination of mobile phase and columns were proposed with minimum injection volume and short run time. Methods were also validated according to ICH guidelines with several parameters namely specificity, accuracy, precision, linearity, linear range, limit of detection, limit of quantitation, ruggedness and robustness. All parameters showed excellent results within the limit. The summary of all mobile phase, column and applications were tabulated in Table 2.[10, 15–25]

Table 2: Important parameters for the determination of Bendamustine using HPLC.

Mobile phase	Stationary phase	Flow rate (ml/min)	Detector (nm)	Application	Reference
Acetonitrile, water and acetic acid (200:50:0.05, v/v/v)	Tracel Excel C_{18} (4.6×250 mm, 5µm)	1.0	233	Drug formulations	[15]
Methanol: water (50:50, v/v)	ODS C_{18} (4.6x250 mm)	1.0	232	Pharmaceutical formulations	[16]
Mobile phase A (0.1% trifluroacetic acid in water) and acetonitrile (90:10, v/v); Mobile phase B (0.1% trifluroacetic acid in water and acetonitrile (50:50, v/v)	Zorbax SB-C_{18} (4.6x25 cm, 5µm)	1.0	230	Bulk drug	[17]
Water and Acetonitrile (with	Zorbax poroshell	0.5	254	Formulations	[18]

0.01% TFA) = 50:50, v/v	120EC C_{18} RP (100x4.6 mm, 2.7 mm)				
Phase A (water and trifluoroacetic acid = 1000:1, v/v); mobile phase B consisting of acetonitrile	Inertsil ODS-2 (250×4.6 mm, 5 mm)	1.0	233	Drug	[10]
Acetonitrile-10 mM potassium dihydrogen phosphate, pH 2.5 (32:68, v/v)	Agilent TC C18 (4.6×250 mm, 5 µm)	1.0	Excitation = 328 Emission = 420	Human plasma and urine	[19]
Mobile phase A (NaH_2PO_4, 10 mM, pH 3.0) and mobile phase B (Acetonitrile)	Synergi Hydro (250×4.0 mm,4 µm),	0.75	Excitation = 330 Emission = 420	Human plasma	[20]
Buffer, pH 7: methanol	ACE C_{18} (250 × 4.6 mm, 5 µm)	1.0	235	Pharmaceutical formulations	[21]
Acetonitrile–water, pH 2.7 with OPA (25:75, v/v)	Synergi Max (150×4.6 mm,4 µm),	1.0	Excitation = 328 Emission = 420	Human plasma and urine	[22]
Sodium salt of octane sulfonic acid (5mM) in methanol, water and glacial acetic acid (55:45:0.075, v/v/v), pH 6	C_{18} Purospher®STAR (250×4.6 mm, 5 µm)	1.5	233	Rat plasma	[23]
Trifluoroacetic acid and acetonitrile (68:32, v/v)	Inertsil ODS -2 (150 x 4.6mm, 5 µm)	1.5	230	Pharmaceutical formulations	[24]
Potassium phosphate dibasic buffer (pH 7) and acetonitrile (70:30, v/v)	Thermo Hypersil C_{18} column (4.6x250 mm, 5µm)	1.0	232	Bulk drug and formulation	[25]

High Pressure Liquid Chromatography and Mass Spectrophotometer (HPLC–MS)

LC-MS/MS method was proposed by Dubbelman et. al. for bendamustine in human plasma and urine sample. LC-MS/MS instrumentation setting was first optimized and studied varied sample preparation technique. The method successfully evaluated assay of the present HP2, M4 and M3 metabolites. To our best knowledge, it was the first validation of bendamustine compound with respect to bioanalytical assay.[26] Li Ding et. al. identified two impurities generated after degradation in bendamustine dosage form. With the help of preparative HPLC impurities were separated and confirmed by NMR.[27] The investigation proposed to keep the bendamustine drug product to be in dark place at room temperature. Pharmacokinetic studies of mice and dog plasma samples were also conducted and validated as per regulatory guidelines. The results were reproducible enough to say about method sensitivity and accuracy.[28] The results from mice dried blood spots gave confirm indication that these pharmacokinetic studies.[29] can be useful for human blood sample routine analysis and in clinical practice to monitor therapeutic action of bendamustine drug. Detailed information of all LCMS procedures for determination of bendamustine in biological fluids available is given in Table 3.

Table 3. Determination of bendamustine in biological fluids using MS.

Mobile phase	Stationary phase	Flow rate (ml/min)	Application	Reference
Gradient programme Mobile Phase A= Ammonium formate with 0.1% formic acid in water (5 mM) Mobile Phase B= Methanol	Synergi Hydro (150 mm×2 mm, 4 µm)	0.25	Human plasma and urine	[26]
Water (pH =2.6 with trifluoroacetic acid) and methanol = 70:30, v/v	Agilent SB C_{18} (150×4.6 mm, 3.5 µm)	0.2	Drug product	[27]
0.2 % formic acid and acetonitrile (25:75, v/v)	Atlantis dC_{18} (50×4.6 mm, 5 µm)	0.4	Mice and dog plasma	[28]
0.2 % formic acid and acetonitrile (25:75, v/v)	Atlantis dC_{18} (50×4.6 mm, 3 µm)	0.5	Mice blood	[29]
Gradient programme Mobile Phase A= 0.1% formic acid Mobile Phase B= Acetonitrile	Luna C_{18} (50×2.1 mm, 1.6 µm)	0.4	Plasma and urine	[30]

CONCLUSION

The most common leukemia disease in adults is chronic lymphocytic leukemia. Generally, it depends on biological activities and fitness ability of the patient. The alkylating agent, bendamustine hydrochloride is very useful for its treatment. The importance of the said drug is not only limited with CLL, it is a valuable medication for multiple myeloma and Hodgkin's lymphoma. Two phases, one through benzimidazole/butyric acid and other mechlorethamine moiety form the metabolites in human, namely hydroxybendamustine, *N*-desmethylbendamustine and mercapturic acid, mercapturic acid with respective pathway. Two hydrolysis products were also formed due to degradation and five possible impurities expected and detected for bendamustine hydrochloride. The main objective of our review is to discuss about analytical methods available for bendamustine and its impurities in bulk, dosage form and biological fluids. The literature will give a brief idea about bendamustine to the new researcher and academician. The instruments which can be used for its determination are UV-visible spectrophotometer, HPLC, LC-MS and UPLC-MS. In the future, very less cost titrimetric method as well as capillary electrophoresis, HPTLC, spectrofluorometric techniques can be useful for its determination.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

AUTHORS CONTRIBUTIONS

Ahmed Abu Judeh: Helped in literature searches, wrote the first draft and approved the final version to be submitted.

SK Manirul Haque: Formulated the study, contributed in the first draft, communicated with the journal and approved the final version to be submitted.

Sreekumar P.A: Helped in the literature survey, extensively reviewed the first draft and provided information for improvement and approved the final version to be submitted.

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