

# Intrinsic stability and degradation pathways of bendamustine in injectable solution



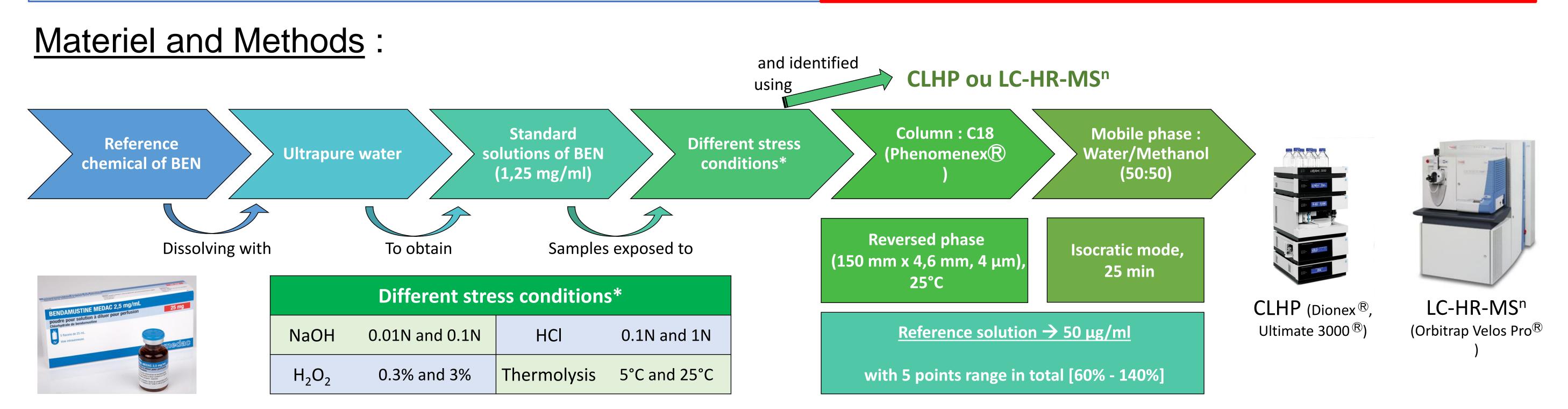
240-65

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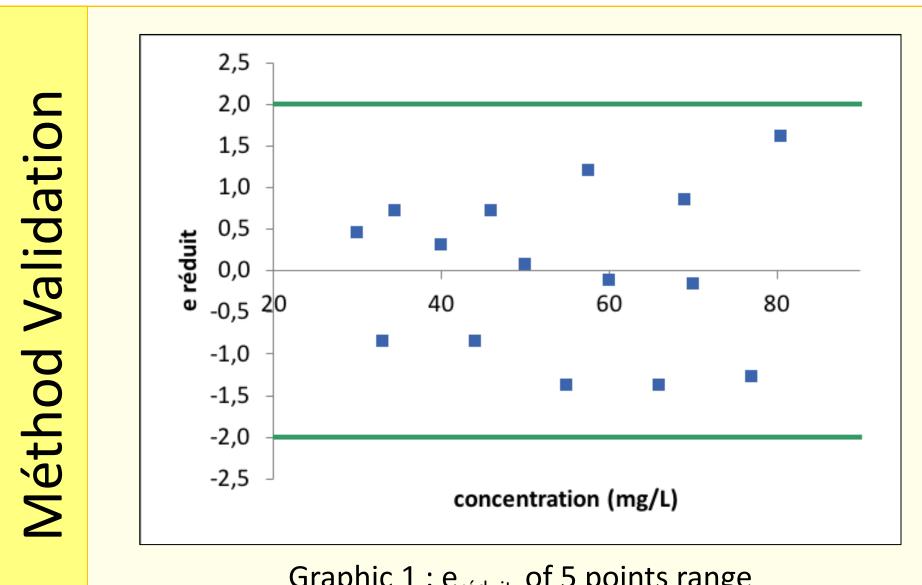
### Introduction:

- Bendamustine (BEN) is a chemotherapy medication used for the treatment of chronic lymphocytic leukemia (CLL), indolent B-cell non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM).
- The stability data provided by the manufacturer stated 8h at 4°C for the lyophilized powder reconstituted with sterile water, 48h and 3h30 for the diluted solution in sodium chloride respectively at 4°C and 25°C.
- As recommended by the International Council for Harmonisation (ICH), studies on a drug must be undertaken to establish the identification of its possible degradation products (DP) and for understanding the intrinsic stability of the drug molecule.

The aim of this study was the assessment of the inherent stability characteristics of BEN under different stress conditions by using Liquid Chromatography-Multistage Mass Spectrometry along with high-resolution Mass Spectrometry (LC-HR-MS<sup>n</sup>).



## Results:





#### LINEARITY

 $y = 0.8272x - 2.4778 (x = [X] in \mu g/ml)$  $r^2 = 0.98386$ 

Graphic 1 of reduced residues is correct because the 3 validation criteria are true:

1/ No represent a structure 2/ It has as much positive as negative (8 against 7), 3/100% of the  $e_{réduit}$  betwenn [-2; +2]

#### REPEATABILITY

Coefficient variability of repeatability CR = 3.3% Intermediate Fidelity IF = 3.0%

	<u>A</u> (	CCUR	<u>ACY</u>		
[X] ( <u>µg/ml)</u>	30	40	50	60	70
Accuracy (%)	3.65	2.30	0	3.14	3.39

Average recovery rate **R** = **99.6** 

[M+H]+ = 362.123367 Da

C<sub>15</sub> H<sub>2</sub> CIN<sub>2</sub> O<sub>6</sub>

Fragmentation	Ionic mass observed (m/z)	Empirical formula	Exact theoretical mass (m/z)	Error (ppm)		
BEN	358,1075	$C_{16}H_{21}CI_2N_3O_2$	358,1084	-2,4		
Α	340,0975	$C_{16}H_{19}CI_2N_3O$	340,0978	-0,9		
В	322,1314	$C_{16}H_{20}CIN_3O_2$	322,1317	-0,9		
С	312,1024	$C_{15}H_{19}CI_2N_3$	312,1029	-1,5		
D	304,1207	$C_{16}H_{18}CIN_3O$	304,1211	-1,4		
Е	276,1257	$C_{15}H_{18}CIN_3$	276,1262	-1,8		
F	268,1440	$C_{16}H_{17}N_3O$	268,1444	-1,6		
G	240,1491	$C_{15}H_{17}N_3$	240,1495	-1,8		
н	228,1135	$C_{13}H_{13}N_3O$	228,1131	1,6		
ı	212,1179	$C_{13}H_{13}N_3$	212,1182	-1,5		
J	200,1180	$C_{12}H_{13}N_3$	200,1182	-1,1		
Table 1. Structures and UD MS date of the major						

Table 1: Structures and HR-MS data of the major fragment ions of BEN

362,1

Ionic mass observed (m/z)

362,1224

326,1474

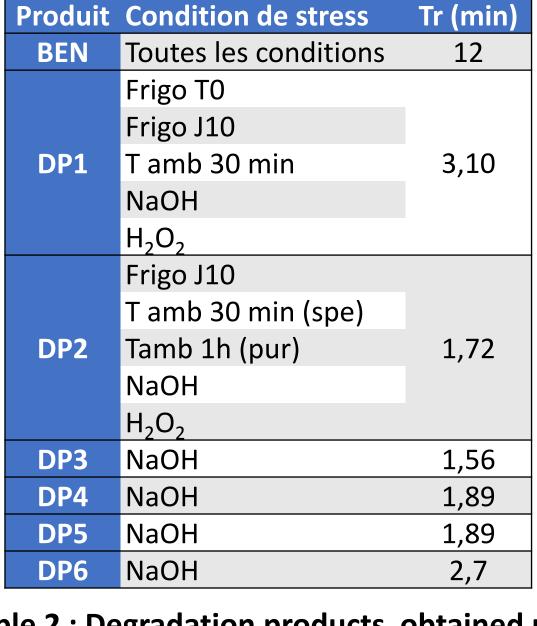


Table 2: Degr stress conditie

$11_2O_2$		+ OH-/H O	
Frigo J10		2	
T amb 30 min (spe)			
Tamb 1h (pur)	1,72	$H_3$ C $H_3$ C $\dots$	_ H+
NaOH		BEN OH DP1 OH	
H <sub>2</sub> O <sub>2</sub>			
NaOH	1,56	- HCI + HO	
NaOH	1,89	[M+H]+= 358.108359 Da	
NaOH	1,89	$ \begin{pmatrix} C_{16} & C_{16} & C_{10} $	
NaOH	2,7	CI 16 21 2 3 2	
gradation product	s obtained u	under - HCI	
.: <b>.</b> . <b>.</b> !			
tions and their ret	tention time	H₃C ₩	
tions and their rei	tention time	H C H <sub>3</sub> C	_ H+
tions and their rei	tention time	H <sub>3</sub> C DP3/DP4 OH OH	H+
eoretical mass (m/z)	Error (ppm)	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	<b>∏</b> H+
		DP3/DP4  HOH-/H <sub>2</sub> O  HOH-/H <sub>2</sub> O  H <sub>3</sub> C  N  H <sub>2</sub> C  N  M+H]+ = 304.165568 Da	_ H+
neoretical mass (m/z)	Error (ppm)	DP3/DP4  H <sub>3</sub> C  OH  H <sub>2</sub> C  H <sub>2</sub> C  N  OH  H <sub>2</sub> C  N  OH  OH  OH  OH  OH  OH  OH  OH  OH	_ H+
neoretical mass (m/z) 362,1234	Error (ppm) -2,7	DP3/DP4  H <sub>3</sub> C  H <sub>4</sub> C  H <sub>2</sub> C  H <sub>3</sub> C  OH  OH  OH  OH  OH  OH  OH  OH  OH  O	H+
secretical mass (m/z) 362,1234 326,1467	Error (ppm) -2,7 2,2	DP3/DP4  H <sub>3</sub> C  H <sub>2</sub> C  OH  OH  OH  OH  OH  OH  OH  OH  OH  O	<b>⊣</b> H+
eoretical mass (m/z) 362,1234 326,1467 308,1361	Error (ppm) -2,7 2,2	DP3/DP4  H <sub>3</sub> C  H <sub>3</sub> C  H <sub>4</sub> C  H <sub>2</sub> C  OH  OH  OH  OH  OH  OH  OH  OH  OH  O	<b>∏</b> H+
eoretical mass (m/z) 362,1234 326,1467 308,1361	Error (ppm) -2,7 2,2 1,9	DP3/DP4  H <sub>3</sub> C  H <sub>2</sub> C  OH  OH  OH  OH  OH  OH  OH  OH  OH  O	

DP6

308,1367  $C_{15}H_{19}N_2O_5$ Table 3: Different fragmentation products fro DP6 CHNO Figure 1 : DP6 fragmentation pathway

**Empirical formula** 

 $C_{15}H_{22}CIN_2O_6$ 

 $C_{15}H_{21}N_2O_6$ 

**Exact the** 

# Discussion:

Fragmentation

DP6

DP6

<u>0</u>

Examp

- **BEN**  $\rightarrow$  fragile under basic, hydrolysis, temperature and oxidative conditions.
- Based on the knowledge of its fragmentation pattern, up to six degradation products (DP1 to DP6) were highlighted suggesting that the degradation of BEN occur via multiple reaction pathways among which, hydrolysis, eliminations, nucleophilic additions or N-dealkylation.

## Conclusion:

Understanding the degradation pathways of BEN was the key factor to mitigate the degradation of the drug product and help to anticipate its degradation.

C<sub>17</sub> H<sub>25</sub> N<sub>3</sub> O<sub>4</sub>

- In view of the degradation of the reconstituted BEN solution, measures should be taken to ensure compliance to good manufacturing practices during the reconstitution, dilution, storage, transport and administration of the drug.
- Finally, the absence of degradation product under acidic condition has to be pursued to improve the drug stability.