WHAT HAPPENS WHEN INSULIN ASPART IS DILUTED IN DEXTROSE ?

Préta L-H.[1], Henry H.[1,2], Masse M.[1,2], Carta N.[1], Kouach M.[1], Foulon C.[1], Goossens J-F.[1], Lannoy D.[1,2], Genay S.[1,2], Décaudin B.[1,2], Odou P.[1,2]

[1] EA 7365 - GRITA - Groupe de Recherche sur les formes Injectables et les Technologies Associées, Faculté de Pharmacie, Université de Lille, Lille, France
[2] CHU Lille, Institut de Pharmacie, Lille, France

Poster n° 3PC – 004
ATC code : A10

http://www.eahp.eu/24-3PC-004

BACKGROUND

In hospital, medications for infusion are mostly diluted in saline. In neonatal resuscitation, glycaemic instabilities are frequently observed in the premature newborns, hence insulin treatment is started. In our establishment, insulin aspart is used and diluted in a 5% dextrose solution (D5%), due to sodium restrictions in newborns.

PURPOSE

To evaluate the impact of the choice of D5% diluent on the stability of the insulin aspart at 1 U/mL.

METHODS

The pharmaceutical specialty composed of insulin aspart and its two preservatives (phenol and metacresol) was diluted in saline or D5% at the concentration of 1 U/mL.

- **Study by an HPLC-UV stability indicating method (Table 1) of :**
  - the impact of the diluent (nature, pH) on the stability of insulin aspart
  - forced degradations (strong acidity & basicity, heat, oxidation)
  - the kinetic of a new compound's appearance by relative evaluation of HPLC-UV signals (n = 4) during 1 week
- **Study by HPLC-MS in full scan mode (Table 2) of :**
  - the prospective formation of a new compound in the different diluents

Equipment: 1260 Infinity Agilent® chain, Agilent Technologies	
Settings	**Conditions**
Stationary phase	C18 ; 2,6 µm, 100 x 2,1 mm
Mobile phase	Ultrapure water / Acetonitrile / Anhydrous sodium sulfate / 85% o-phosphoric acid (77 / 21 / 1,8 / 0,2 ; m/m)
Flow-rate	0,3 mL/min
Column temperature	25 °C
Injection volume	100 µL
Wavelength	214 nm

Table 1 : HPLC-UV conditions

Equipment: HPLC : UFLC-XR, Shimadzu® - MS : API QTrap 5500, AB Sciex®	
Settings	**Conditions**
Stationary phase	C18 ; 5 µm, 100 x 2,1 mm
Mobile phase	Ultrapure water / Acetonitrile (80/20 ; v/v) and 0,1% Formic acid
Flow-rate	0,4 mL/min
Ionisation source	Electrospray in positive mode ionization
Injection volume	10 µL

Table 2 : HPLC–MS conditions

RESULTS

- In saline, there were 3 signals in HPLC-UV **(Figure 1)**
- After dilution of insulin aspart in D5%, a fourth signal appeared **(Figure 2)**
- pH influence and forced degradation tests failed to attribute this signal to insulin or preservatives degradation.
- HPLC-MS analysis revealed a mass difference of **162 daltons** between insulin and this product, which corresponds to a **glycation phenomenon** of insulin aspart. Indeed, it is known that glycation is the creation of a covalent bond with a **glucose molecule (180 Da)** and the departure of the equivalent of a **water molecule mass (18 Da)**
- Finally, the kinetics showed that insulin glycation phenomenon seems to increase with the contact time between insulin and glucose until a plateau is reached after 24h of contact **(Figure 3)**.

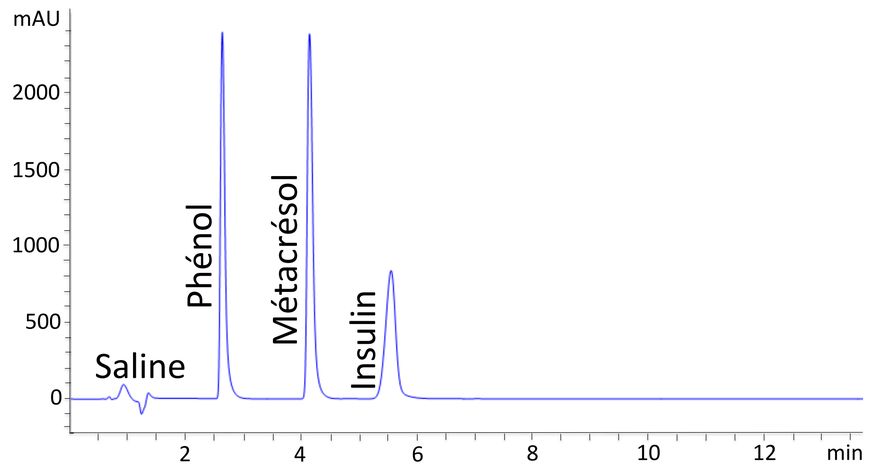

Figure 1 : Insulin 1 U/mL in saline

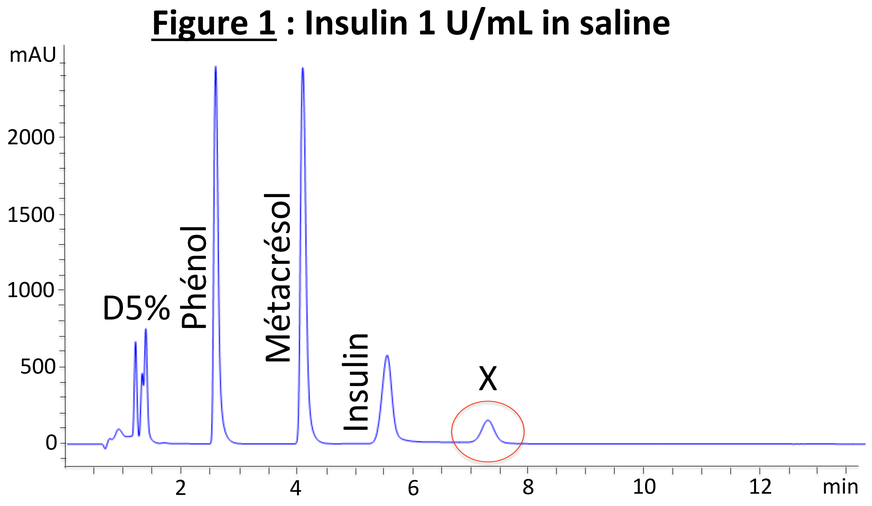

Figure 2 : Insulin 1 U/mL in D5%

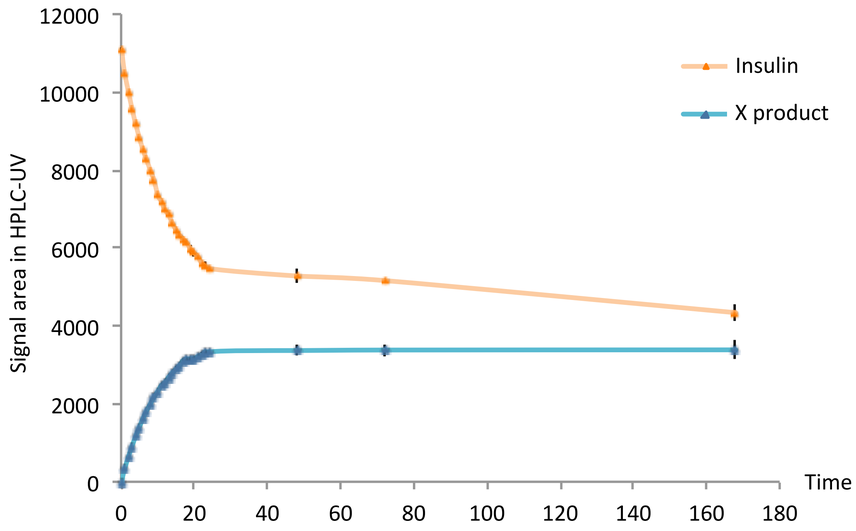

Figure 3 : Evolution of signals areas for insulin and glycated insulin expressed in average areas ± standard deviations (n = 4)

DISCUSSION - CONCLUSION

This work highlighted the instability of insulin in D5% and showed the phenomenon of insulin aspart glycation. To better characterize this phenomenon, the biological effect of glycation on insulin activity have to be determined, since a decrease in activity has been observed for human insulin.

Bibliography :
- Poulsen & al. , 2008 , *Pharmaceutical Research*
- O'Harte & al. , 1996 , *Peptides*
- Hunter & al. , 2003 , *Diabetes*