

# The stability of soluble insulin in plastic syringes

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## ABSTRACT

**Background:** A ready-to-use (RTU) syringe of insulin 1 IU/mL was developed by the hospital pharmacy to improve its safe administration and limit the risk of confusion with heparin in intensive care units (ICUs). The syringes were prepared under aseptic conditions according to good manufacturing practice.

**Study objectives:** To establish the stability of insulin 1 IU/mL in 0.9% sodium chloride in the RTU syringes.

**Methods:** The chemical stability of insulin solution 1 IU/mL in 0.9% sodium chloride was determined at 4°C, 25°C and 40°C by a stability-indicating liquid chromatography method. The pH was measured throughout the study and the presence of particulates was controlled. Sterility testing was performed to test the integrity of the syringes.

**Results:** Solutions were judged to be stable if the drug level remained at more than 90% of the initial concentration. After one month, insulin levels were below 80% for syringes stored at 40°C and above 95% for syringes stored at 4°C and 25°C. After three months, syringes at 25°C showed an insulin concentration below 90% and after six months, the concentration of insulin contained in syringes stored at 4°C was still above 90%.

The pH did not change appreciably during the study and the sterility testing results were found to be negative. Moreover, in all cases, the syringes fulfilled all *European Pharmacopoeia* criteria in terms of non-visible particles.

**Conclusion:** RTU syringes supplied by the hospital pharmacy and stored in the ICU unit were found to be stable at 4°C–8°C for six months and for one month at room temperature, with no significant loss in potency.

## KEYWORDS

Central intravenous additive service (CIVAS), drug stability, insulin, intensive care units (ICUs), polypropylene syringes

## INTRODUCTION

In 2001, a study by Van de Berghe et al. showed that tight glycaemic control (TGC) and intensive insulin therapy (IIT) reduced mortality among patients who remained critically ill for a prolonged period [1]. Based on their results, TGC became standard care in most intensive care units (ICUs). However, more recent studies have demonstrated a higher risk of hypoglycaemia when TGC and IIT protocols are used, increasing mortality among these patients. Therefore, practice evolved from an intensive (targeted blood glucose: 4.5–6.0 mmol/L) to a more conventional glucose control (targeted range: 6–8 mmol/L) in critically ill patients [2–4].

The targeted blood glucose level has changed in recent years; nevertheless, administration of continuous insulin

remains common practice in ICUs, involving the frequent preparation of insulin infusions [4].

Between 2002 and 2005, 42 incidents related to the administration of insulin and heparin were reported in the University Hospitals of Geneva (HUG). These two drugs have sound-alike names, are contained in look-alike vials and necessitate similar preparation. The latter two facts are the likely reason for the high number of reported incidents.

Although the patients suffered no long-term ill effects, the risk of confusion between the two products needed to be addressed in order to limit any further critical events.

Several studies have demonstrated that diluted insulin solutions in syringes can be stored for a few days under refrigeration [5–7]. However, as far as is known, no data have been published showing stability for longer than one month.

The aim of this study was to develop ready-to-use (RTU) syringes of insulin 1 IU/mL, with a long shelf-life and prepared in batches under aseptic and good manufacturing practice (GMP) conditions, in order to improve its safe administration and limit the risk of confusion with heparin.

## MATERIAL AND METHODS

### Preparation of the syringes for stability studies

A solution of human insulin 1 IU/mL in 0.9% sodium chloride was prepared by an appropriate dilution of Actrapid HM

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Received: 12 May 2010; Revised manuscript received: 26 August 2010; Accepted: 1 September 2010

100 IU/mL from Novo Nordisk (Bagsvaerd, Denmark). Because Actrapid HM already contains stabilising agents, i.e. metacresol and zinc sulphate, only water for injection was used for dilution. The solution was filtered (0.22 µm) and 50 mL were transferred into 60 mL PlastiPak syringes comprising a polypropylene barrel, polypropylene/polyethylene plunger and synthetic isoprene free of natural rubber and latex plunger gasket, silicone oil as lubricant (Becton Dickinson, NJ, USA) under a laminar-airflow hood in a GMP class B clean room. The syringes were sealed using tamper-evident caps (TEC 1000, B. Braun, Melsungen, Germany) and individually packed into plastic sachets. They were stored at 4°C ± 2°C, 25°C ± 2°C and 40°C ± 2°C. The solutions were assayed immediately after preparation of the syringes (day 0) and on day 7, day 30, month 2, month 3 and month 6.

### High-performance liquid chromatography (HPLC) analysis

#### *Chromatographic equipment and conditions*

The quality control laboratory of the pharmacy department at HUG developed a method by adapting the one given in the *European Pharmacopoeia* [8].

The HPLC system was a Merck-Hitachi LaChrom D7000 HSM (Merck, Darmstadt, Germany) equipped with an L-7200 autosampler, an L7100 high pressure pump and an L-7455 diode array detector. Separations were carried out on a Lichrosorb RP-8 column 125 x 4.6 mm internal diameter with a particle diameter of 5 µm (Merck). The mobile phase consisted of 28.4 g/L sodium sulphate adjusted to pH 2.3, acetonitrile (Merck) and 2-methoxyethanol (Fluka, Buchs, Switzerland) 770:274:6. The flow rate was 1 mL/minute, the detection was monitored at 212 nm and the temperature was ambient. The injection volume was 20 µL.

#### *Validation of the HPLC method*

The chromatographic method was validated according to *Société Française des Sciences et Techniques Pharmaceutiques* guidelines before applying it to the stability study of the RTU human insulin solution [9].

Standard (std) and reconstituted formulation (rf) solutions at five insulin concentration levels (0.5, 0.75, 1, 1.25 and 1.5 IU/mL) were prepared daily and used to test linearity and trueness. Six different rf solutions at 1 IU/mL corresponding to 100% of insulin present in the formulation were prepared each day and used to determine the precision of the method. Slopes and intercepts were not significantly different for std and rf samples (t tests). All the statistical tests were positive, thus verifying the linearity

and trueness of the method. Trueness values between 97.8% and 100.9% were obtained with repeatability and intermediate precision values of 1.4% and 2.1%, respectively. Correlation coefficients of 0.9989 for std and 0.9995 for rf solutions were obtained. Thus, the method was accepted for determining insulin concentrations in RTU IV plastic syringes.

#### *Stability indicating method*

To rule out possible interference of degradation products during the insulin determination, 1 mL of both a 1 N sodium hydroxide solution and a 1 N phosphoric acid solution were added to 1 mL of the insulin solution, respectively. The solutions were heated at 70°C in a water bath for 30 minutes and then diluted before HPLC injection. The absence of degradation products eluting under the analyte peak was checked by comparing the peak height/area ratio for insulin at two different wavelengths (212 and 254 nm) with insulin standard (except when no peak was observed).

#### *Sample preparation during the stability study*

Actrapid HM 100 IU/mL (Novo Nordisk, Denmark) was used as the insulin stock solution. A stock solution of phenol 0.1% (m/v) (internal standard) was prepared in distilled water. All stock solutions were extemporaneously prepared.

Standard samples were prepared by diluting the stock solutions in water to obtain an insulin concentration range of 0.5–1.5 IU/mL and a phenol concentration of 0.002%.

For insulin samples, 200 µL of the stock solution of phenol 0.1% was added to 10 mL of insulin assay solution 1 IU/mL in the syringe.

Three different syringes were used for analyses at each time interval.

### **Sterility testing**

The tests were performed using a method developed by the quality control laboratory in the pharmacy department at HUG [10]. Two syringes at both temperatures were tested for sterility at the beginning and end of the study.

### **pH determination**

The pH of the solutions was measured at each time interval with a glass electrode pH meter (Metrohm model 691, Herisau, Switzerland).

### **Particulate matter**

A HIAC Royco counter with a HRLD-50 sensor module from Skan (Basel, Switzerland) was used for particle count

determinations. Four runs were carried out and the results of the first run were discarded.

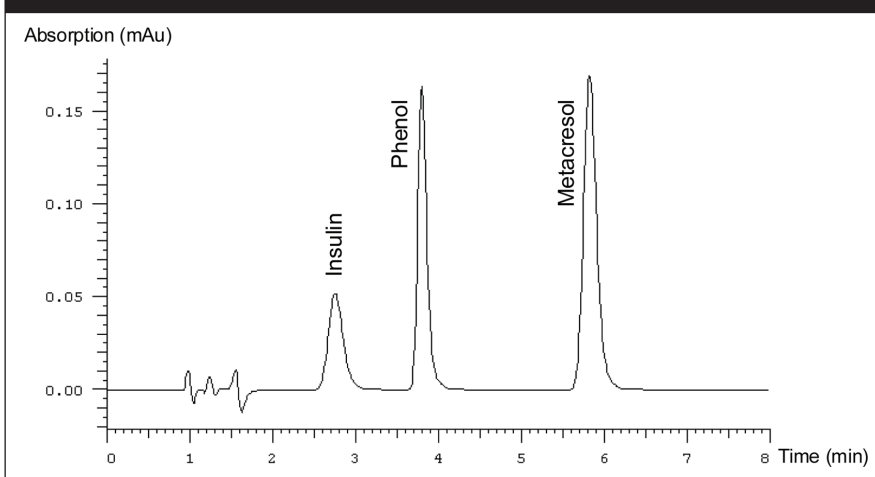
### Endotoxin detection

The tests were performed using the method for endotoxin as recommended in the *European Pharmacopoeia* [11]. Three syringes were tested (one for each of the temperatures).

### RESULTS

A typical chromatogram obtained from the analysis of insulin solution contained in syringes is shown in Figure 1. The retention times for insulin, phenol and metacresol (preserving agent) were 3.3, 4.2 and 6.7 minutes, respectively.

**Figure 1: LC chromatogram obtained for the LC-UV analysis of insulin 1 IU/mL contained in RTU syringes**



No interference of degradation products during insulin determination was observed with the accelerated degradation test. No insulin peak and no further peak were recorded on LC chromatograms at two wavelengths (212 nm and 254 nm) for the analysis of samples treated with basic and acidic solutions.

Results obtained for the stability study of insulin in RTU syringes are shown in Table 1. The pH did not change appreciably throughout the entire study and the results from sterility and endotoxin tests were found to be negative in all cases. After one month, insulin levels (stable if 90–110%) were under 60% for syringes stored at 40°C and above 95% for syringes stored at 4°C and 25°C. After two months, drug levels were above 95% for syringes stored at 4°C and 25°C. After three months, insulin concentrations of 97% and 85% were obtained for syringes at 4°C and 25°C respectively. Moreover, the syringes stored at 25°C did not fulfil *European Pharmacopoeia* criteria in terms of non-visible particulate matter. Indeed, mean particle values per syringe were 18,150 for 10 µm particles and 1,650 for 25 µm particles. The final step of the study simulated possible storage and use conditions of the syringes. It consisted in the quantification of insulin compound in syringes first stored at 4°C for six months and then at 25°C for one more month. Drug concentrations above 90% were also obtained with satisfactory values of non-visible particulate matter, compatible with IV injection (10 µm: 7,600 and 25 µm: 900).

Moreover, the volume of solution contained in the syringes was checked at each time interval by the operator;

no decrease in volume greater than 1.0 mL (which corresponds to 2% of the total volume) was observed. In other words, no relevant water evaporation had occurred. No significant loss of insulin was observed in syringes stored under refrigeration because of degradation or adsorption of insulin on to containers. Indeed, this well-recognised phenomenon mainly occurs when the concentration of insulin is below 0.2 IU/mL [7].

**Table 1: Insulin concentrations in CIVAS syringes stored at three different temperatures**

Time (day)	Syringes stored at 4°C* (%)	Syringes stored at 25°C* (%)	Syringes stored at 40°C* (%)
0	100	100	100
7	101	105	53
30	98	96	
58	98	95	
90	97	85	
180	93		
After storage of 180 days at 4°C, syringes of insulin were stored at 25°C			
180 days at 4°C + 1 day at 25°C	98		
180 days at 4°C + 7 days at 25°C	93		
180 days at 4°C + 30 days at 25°C	96		

\*Three different syringes tested at each time interval. No inter-syringe variability was observed (data not shown).

## DISCUSSION

To the best of the researchers' knowledge, there is no published paper reporting a long-term stability study of a low concentration solution of insulin for IV administration. Given the interest in RTU insulin to improve administration safety, this study was performed by the hospital pharmacy at HUG.

Once the stability study on insulin was completed, the RTU syringes were rapidly implemented in the ICUs. Insulin vials were retrieved from all the ICUs and replaced by 50 IU/50 mL RTU syringes. Insulin syringes prevent confusion errors during preparation and administration stages; this was the primary motivation for designing these syringes. Indeed, having insulin syringes and heparin vials drastically reduced the risk of confusion. In fact, no further reports involving a mix-up between the two drugs were received after the introduction of the RTU syringes.

Moreover, this method ensures proper aseptic conditions during the preparation of syringes, thereby reducing the risk of microbiological contamination. The RTU syringe also reduced the nurses' workload in the ICU knowing they can connect the RTU syringes directly to the syringe drivers without any other manipulation.

The choice of packaging material was based on the use of polypropylene syringes. This material is considered as a medical device and not packaging material in contact with drugs according to the *European Pharmacopoeia* [12, 13]. However, syringes made from polypropylene are the only multipack sterile syringes commercially available on the Swiss market. Moreover, polypropylene syringes are a simple and safe alternative for this study because polypropylene is commonly used as packaging material for injectable containers (bags and bottles) and it has also been used for many years in the HUG pharmacy for several types of RTU drugs (10 different compounds and over 100,000 syringes produced and injected), with no observed or reported clinical adverse drug reaction.

## CONCLUSION

RTU insulin syringes supplied by the hospital pharmacy and stored in ICU were found to be stable for six months at 4°C–8°C and for one month at room temperature, with no significant loss in potency.

Beside their highly practical use in an ICU setting, implementation of insulin RTU syringes allowed a real improvement in safety during insulin and heparin administration.

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