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Short communication

Shelf lives of aseptically prepared medicines—stability of netilmicin injection in polypropylene syringes

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Abstract

There is no published information on the stability of netilmicin solutions in prefilled syringes. The purpose of this study was to evaluate the stability of netilmicin in polypropylene syringes and to determine the optimum validated shelf life so that they may be prepared in bulk in appropriately licensed facilities.

The syringes containing netilmicin 10 or 100 mg/ml were stored at 7 °C, room temperature in the light (RTL) and 25 °C/60% relative humidity for up to 300 days.

Netilmicin concentration was determined by reversed phase high performance liquid chromatography (RP-HPLC) of the isoindole derivative formed with *o*-phthalaldehyde (OPA). The shelf lives were calculated using the maximum rate method applied to the netilmicin analytical data. At 7 °C 10 and 100 mg/ml solutions were stable for 90 days falling to 30 days at 25 °C and 60% RH. At RTL the 10 mg/ml solution was stable for 9 days.

Keywords: Netilmicin; Netillin; Stability; Shelf life; Aseptically prepared medicines

1. Introduction

Netilmicin (Fig. 1) is a semisynthetic aminoglycoside antibiotic used as an alternative to amikacin in the treatment of infections caused by susceptible bacteria that are resistant to gentamicin and tobramicin. It is used at strengths of between 10 and 100 mg/ml, which may be advantageously distributed into various size syringes in validated, licensed, hospital pharmacy aseptic units for future use.

The storage period for prefilled syringes of products that have been prepared aseptically depends on a number of factors. Data must be assembled which confirms the microbiological integrity of the syringe system during storage and use and that the processes used in preparation do not allow the ingress of initiating organisms. The chemical stability of the product also needs to be confirmed.

For the practice of preparing these dosage forms in aseptic facilities to be viable, large batch production needs to be adopted. Optimisation of the storage

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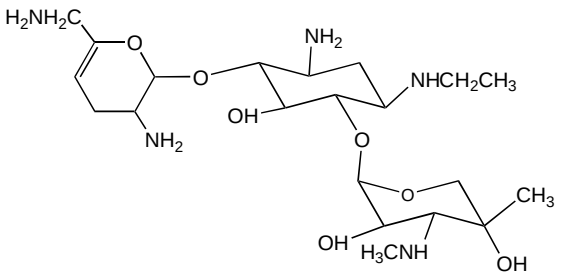

Fig. 1. Netilmicin structure.

life (under refrigeration to minimise microbacterial growth) is therefore necessary.

There are no published stability data available for netilmicin in syringes. The purpose of the present study was to provide such data and thus allow the allocation of a validated shelf life.

2. Experimental

2.1. Materials and reagents

All commercial reagents and materials were obtained from VWR International Ltd. (Lutterworth, England). Netilmicin sulphate (Netillin®) and netilmicin sulphate analytical standard were kindly supplied by Schering-Plough Ltd (Welwyn Garden City, England). Polypropylene 2 ml Omnifix® syringes were obtained from B|Braun (Sheffield, England). Luer-lock syringe caps were obtained from Baxa Ltd (North Ascot, England).

2.2. Apparatus and chromatographic conditions

The high performance liquid chromatographic (HPLC) system (Thermo Electron, Hemel Hempstead, England) consisted of a vacuum degasser, binary gradient pump (P200), autosampler fitted with sample preparation (AS3000) and a UV/Vis detector (UV150). Chromatographic results were collected by data handling software (Scientific Software Inc. EzChrom Elite Version 2.61, Aston Scientific Ltd., Stoke Mandeville, England). Measurements of pH were carried out using a combination electrode pH meter (Corning, model 120, Halstead, England). The chromatographic separation of the thio-substituted isoindole derivatives from the derivatisation with *o*-phthalaldehyde (OPA) in the presence of thioglycollic acid was performed at ambient temperature on a reverse phase Platinum C18 column 150 mm × 4.6 mm i.d., 5 μm particle size (Alltech Associates, Inc. Carnforth, England).

Elution was established with a mobile phase composition of methanol–water–acetic acid (67:28:5, v/v/v) and the ion pairing reagent sodium heptanesulphonic acid (0.2%), at a flow rate of 1.0 ml/min. The chromatographic signal was monitored at 330 nm. The injection volume was 10 ul.

2.3. Method of derivatisation

UV detection of aminoglycosides is severely limited by the absence of a suitable chromophore. Derivatisation yields higher sensitivity. Several methods for the derivatisation of aminoglycosides have been reported in the literature [1–6]. The method used here was an adaptation of that used for the analysis of gentamicin [6], a similar aminoglycoside antibiotic. It involves heating with the derivatising reagent at 60 °C for 15 min. The derivatising reagent consists of 400 mg of OPA in 2 ml methanol, 38 ml borate buffer pH 10.4 and 0.8 ml thioglycolic acid, the whole adjusted to pH10.4. OPA reacts with the primary amine groups in the presence of a thiol to give a 1-alkylthio-2-alkyl substituted isoindole. The derivatisation was carried out using the sample preparation function of the autosampler.

2.4. Standard and sample solutions for HPLC analysis

Stock standard solutions of netilmicin sulphate in water at a nominal concentration of 1 mg/ml netilmicin free base were further diluted in water giving a concentration of 0.4 mg/ml. Derivatising reagent (1.6 ml) was added to 4 ml of this solution and diluted with methanol to give a nominal concentration of 0.16 mg/ml netilmicin. This solution was heated for 15 min at 60 °C before injection. Two independent standards were prepared at each time point. All dilutions and derivatisations were carried out using the autodilution and sample preparation function of the autosampler. Samples were diluted in water and derivatised in the same way to give a nominal injection concentration of 0.16 mg/ml netilmicin, in line with the standard.

2.5. Thin layer chromatography (TLC)

TLC was used as a complimentary technique to HPLC, in order to monitor the appearance of decomposition products. The method [7] utilised a silica gel plate R developed over 10 cm using a mixture of concentrated ammonia–methanol–dichloromethane (20:40:40 v/v/v). The developing solvent was removed in a current of warm air, the plate viewed at $\lambda = 254$ and 366 nm, then sprayed with ninhydrin and stannous chloride reagent R and heated at 110 °C for 20 min. A reference zone of Netillin® injection containing 0.2 mg of netilmicin was similarly treated.

2.6. Preparation of netilmicin Injections

Netillin® injections at strengths of 10 or 100 mg/ml were pooled to achieve homogenous solutions which were each distributed into several syringes and capped.

2.7. Stability study protocol

Syringes containing 10 and 100 mg/ml netilmicin were stored at each of 7 ± 1 °C, 25 ± 2 °C/60% relative humidity (RH) ±5% and at room temperature in the light (RTL). 7 °C was chosen to reflect the highest temperature the syringes were likely to be exposed to in a refrigerator conforming to the ICH guideline [8] for refrigerated storage. 25 °C/60%RH is the ICH guideline condition for long term stability testing and is representative of room temperature storage. The RTL condition (room temperature exposed to continuous irradiation from daylight fluorescent tubes) was chosen to reflect the conditions that the syringes may be exposed to on a hospital ward.

At each time point two syringes were tested for pH, netilmicin content, appearance of decomposition products by TLC, and appearance of the solution and container.

2.8. Calculation of shelf life

The shelf life was calculated using the confidence bound, or maximum rate method [9]. The slope of the least squares linear regression line of ln (concentration) versus time represents the rate constant (k) of the decomposition. The regression line was constructed using all the individual assay values.

The maximum rate method calculates the upper confidence bound of this rate of decomposition, which thus corresponds to the maximum rate of decomposition represented by the analytical data.

A validated Excel™ spreadsheet was used to calculate the shelf lives based on an acceptable loss of 10% of the initial concentration (t_{90}).

For samples where no decomposition was observed throughout the study period an arbitrary maximum time allowed for aseptically prepared syringe storage was applied, i.e. 3 months refrigerated.

3. Results

3.1. Validation of the analytical method

One large peak was obtained in both sample and standard chromatograms at 5.97 min, thought to be derivatised netilmicin (Fig. 2). The unresolved group at 2–3 min is due to by-products of the derivatisation process, confirmed by chromatographing a blank, prepared by omitting netilmicin from the sample.

A derivatised standard was repeatedly injected over a period of 24 h. The peak area of netilmicin decreased with time while the peaks at 3.02 and 4.24 min increased, implying that these two peaks are a result of decomposition of the derivatised netilmicin. It is well documented [10] that OPA derivatised samples are unstable and so the time taken for the netilmicin derivative peak area to fall by two times the method standard deviation was calculated. It was concluded that no derivatised standard/sample should be used after 2 h.

In order to determine whether the proposed method could separate netilmicin from its degradation products or impurities, netilmicin solutions containing 0.5 mg/ml were heated in acid and base for up to 166 h. The sample in acid (Fig. 3) showed a decrease in netilmicin peak area and an increase in peaks at 2–3 min. A new peak was observed at 5 min and an extra zone at Rf = 0.30 was observed in the TLC with ninhydrin and stannous chloride reagent. The originally water-white sample had become yellow. It is well known that glycosidic bonds, present in aminoglycosides can be broken by hydrolysis with acid [11], and it is possible that the peak at 5 min

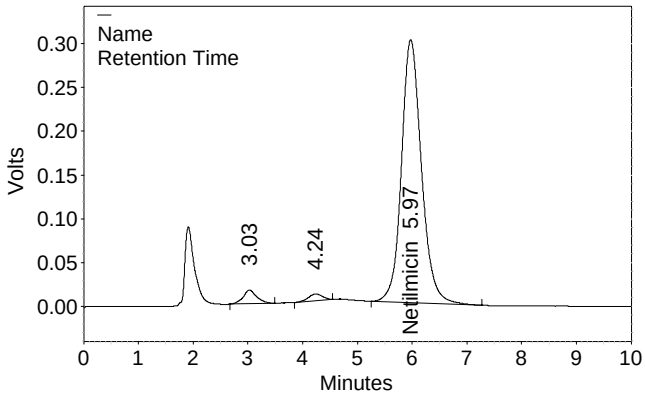

Fig. 2. Chromatogram showing derivatised netilmicin.

could be the OPA derivative of the nitrogen moiety.

The base treated sample showed a slight decrease in netilmicin peak area and a slight increase in the area of peaks at 2–3 min.

3.1.1. Precision, accuracy, recovery and linearity

The method precision (repeatability) was established by assaying six replicates of sample. Intermediate precision was carried out using a different HPLC instrument on a different day. The overall precision was 2.02% R.S.D. ($n = 12$).

The accuracy of the assay system was assessed by spiking a sample of Netillin® with 20, 50, 75, 100 and 125% of netilmicin sulphate. The average recovery at the 100% level was 99.4%, calculated from the equation:

$$\left[\frac{\text{actual amount found (mg/ml)}}{\text{theoretical amount (mg/ml)}}\right] \times 100$$

The linear regression analysis of the dependence of the amount found in mg/ml (y) on the amount added in mg/ml (x) in the accuracy determination gave the equation $y = 0.9486x + 0.9512$, with a correlation coefficient of 0.9990.

3.2. Results of stability study

When stored at 7 °C (Tables 1 and 2) neither 10 or 100 mg/ml solutions of netilmicin exhibited measurable decomposition by assay, or appearance of decomposition product zones in TLC. No change

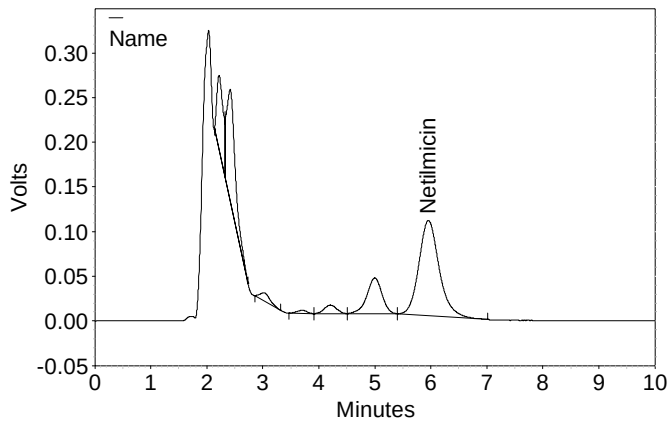

Fig. 3. Chromatogram showing acid decomposed netilmicin after derivatisation.

Table 1
Assay and pH results of netilmicin 10 mg/ml at 7 °C

Time (days)	mg/ml ($n = 2$)[a]	Mean percent of initial remaining	pH
0	10.96, 10.62	100.0	5.69
31	9.91, 10.50	94.6	4.10
42	11.02, 11.19	102.9	4.11
100	10.61, 10.23	97.7	3.98
150	10.69, 10.83	99.7	4.04
200	10.90, 10.99	101.4	3.97
300	10.73, 10.87	100.1	3.97

[a] Data shown are the mean injections of two individual syringe results.

Table 2
Assay and pH results of netilmicin 100 mg/ml at 7 °C

Time (days)	mg/ml ($n = 2$)[a]	Mean percent of initial remaining	pH
0	122.65, 118.09	100.0	5.39
1	120.10, 121.70	100.4	5.26
2	123.45[b]	102.6	5.23
7	121.52, 124.24	102.1	5.18
29	117.34, 118.07	97.8	4.59
65	122.96, 126.73	103.7	4.09
102	129.35, 127.70	106.8	4.11

[a] Data shown are the mean injections of two individual syringe results.
[b] Single sample.

in the appearance of the solution was noted. A storage life of 3 months may be allocated to both solutions.

At 25 °C/60% RH (Tables 3 and 4) solutions at both concentrations exhibited darkening of colour (from colourless to pale yellow at 10 mg/ml after 65 days and from an initial very pale yellow to dark yellow at 100 mg/ml after 102 days). TLC showed the appearance of a fluorescent (366 nm) zone, Rf = 0.60 after 100 and 102 days, respectively. This was not reflected in a significant change in assay value. The solutions were sufficiently stable to allow storage and use at room temperature protected from light for up to 3 months. Microbiological considerations would however almost certainly prohibit this in practice.

The single set of syringes containing netilmicin 10 mg/ml stored at RTL (Table 5) exhibited measurable decomposition. k (first order) $= 9.255 \times 10^{-3}$ per day. The solution changed from clear and colourless to slight yellow during 80 days storage, with the appearance of 2 decomposition product zones in TLC (Rf = 0.30 with ninhydrin spray after 42 days and Rf = 0.60, 366 nm fluorescent after 42 days). The calculated shelf life was 9.6 days (Fig. 4).

Table 3
Assay and pH results of netilmicin 10 mg/ml at 25 °C/60%RH

Time (days)	mg/ml ($n = 2$)[a]	Mean percent of initial remaining	pH
0	10.96, 10.62	100.0	5.69
31	10.42, 10.47	96.8	3.75
42	10.66, 10.58	98.4	3.71
65	10.24, 10.22	94.8	3.68
100	9.93, 10.23	93.4	3.39
150	10.18, 10.44	95.5	3.52
200	10.38, 10.40	96.3	3.40

[a] Data shown are the mean injections of two individual syringe results.

Table 4
Assay and pH results of netilmicin 100 mg/ml at 25 °C/60%RH

Time (days)	mg/ml ($n = 2$)[a]	Mean percent of initial remaining	pH
0	122.65, 118.09	100.0	5.39
1	123.61, 120.00	101.2	5.29
2	117.96, 122.71	100.0	5.15
7	124.33, 123.96	103.1	4.34
29	121.82, 121.10	100.9	3.76
65	124.57, 124.84	103.6	3.49
102	124.78[b]	103.7	3.35
150	120.94, 122.83	101.3	3.07

[a] Data shown are the mean injections of two individual syringe results.
[b] Single sample.

Table 5
Assay and pH results of netilmicin 10 mg/ml at RTL

Time (days)	mg/ml ($n = 2$)[a]	Mean percent of initial remaining	pH
0	10.96, 10.62	100.0	5.69
2	10.88, 10.81	100.5	5.34
31	7.41, 7.42	68.7	2.44
42	7.37, 7.40	68.4	2.43
56	6.35, 6.89	61.3	2.41
80	6.62, 6.70	61.7	2.38
120	5.56, 5.83	52.8	2.32

[a] Data shown are the mean injections of two individual syringe results.

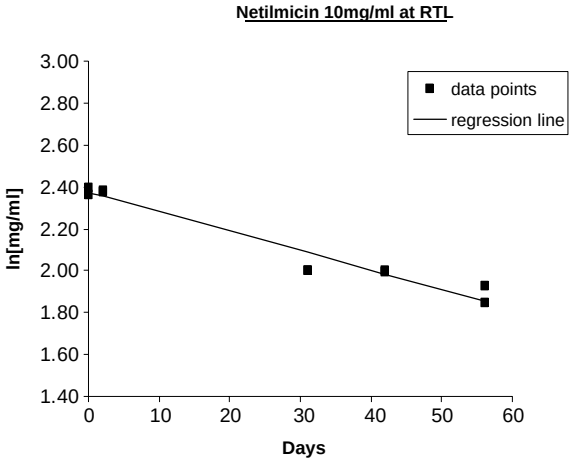

Fig. 4. Example of graph for shelf life determination.

4. Discussion

Whilst HPLC assay results failed to detect significant decomposition under either refrigerated or 25 °C/60%RH storage, the slow deepening of the yellow colour of the solution and the appearance of the two TLC zones under the latter conditions betrayed the presence of incipient degradation.

A detectable change in netilmicin content was noted for the RTL samples. Assay results had fallen to half the original value after 120 days. This fall was accompanied by the appearance of a decomposition product zone in the TLC.

5. Conclusion

Netillin ® injection stored in 2 ml polypropylene syringes at a strength equivalent to 10 or 100 mg/ml netilmicin is stable for up to 90 days if stored at 7 °C. It is stable up to 30 days if stored at 25 °C/60%RH, but only if appropriate microbiologically.

It is stable for up to 9 days if stored at RTL at a strength of 10 mg/ml netilmicin, but should be protected from light wherever possible.

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