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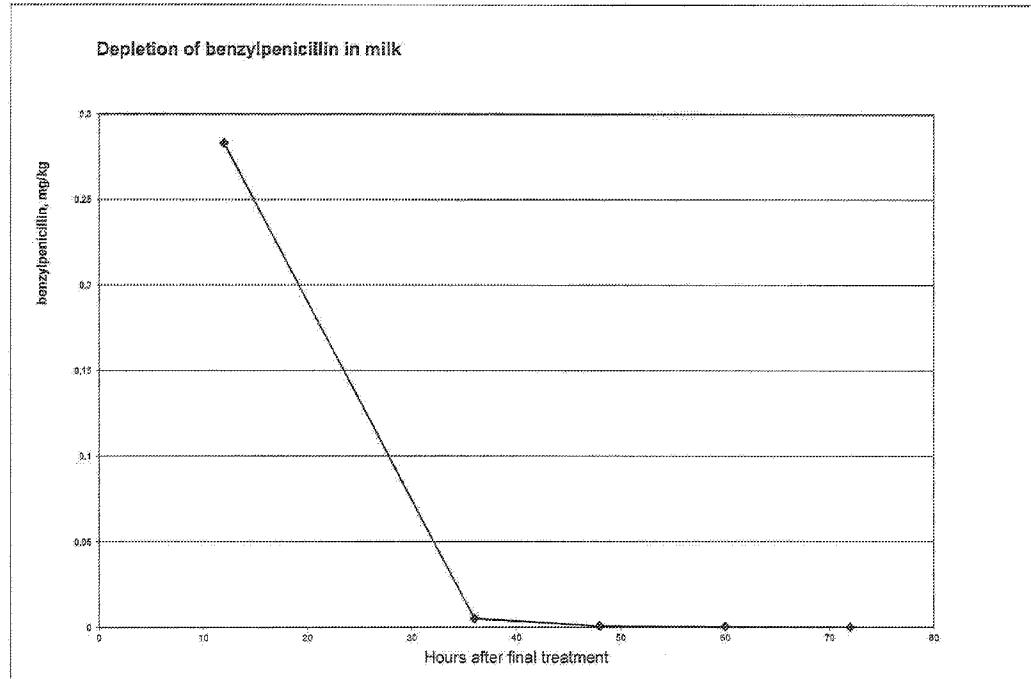
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This invention relates to a composition, including penethamate (PNT) or a pharmaceutical equivalent thereof; and at least one oily vehicle.

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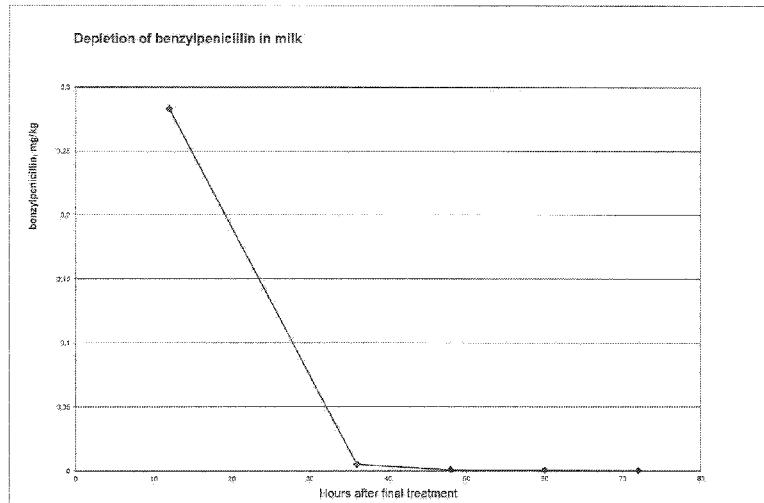
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(54) Title: INJECTABLE ANTIBIOTIC FORMULATIONS AND THEIR METHODS OF USE

FIGURE 16



(57) Abstract: This invention relates to a composition, including penethamate (PNT) or a pharmaceutical equivalent thereof, and at least one oily vehicle.

INJECTABLE ANTIBIOTIC FORMULATIONS AND THEIR METHODS OF USE

TECHNICAL FIELD

The present invention relates to injectable antibiotic formulations and their methods of use,

5 and particularly, but not specifically to the treatment or prevention of mastitis in lactating animals.

BACKGROUND ART

Microbial infections in animals, such as mastitis in dairy cows, are typically treated or

prevented during the lactation period by, intramammary (IMM) infusion, intramuscular (IM) or

10 subcutaneous (SC) injection.

Typically, antibiotics such as penicillins or prodrugs of benzylpenicillin (BP) such as penethamate (PNT) have been used as actives of choice to treat bovine mastitis. PNT is the diethylaminoethyl ester of benzylpenicillin. In formulations intended for veterinary use PNT is incorporated as the hydroiodide (HI). PNT HI is used in intramammary products during

15 lactation (UBRO YELLOW™, Boehringer Ingelheim) and during the dry-off period (UBRO RED™, Boehringer Ingelheim), as well as an injectable suspension during lactation period (Mamyzin™, Boehringer Ingelheim and Penethaject™, Bayer Animal Health) for treatment of mastitis in cows.

PNT is a prodrug from which benzylpenicillin and diethylaminoethanol are released by

20 hydrolysis. Antimicrobial activity of the compound is exclusively related to benzylpenicillin.

The maximum residue limit (MRL) for milk in bovine in e.g. Europe and New Zealand is 4 µg/kg (EMEA, BP).

As a prodrug of benzylpenicillin, penethamate hydroiodide is effective in treating mastitis as

a result of its particular pharmacokinetics. After intramuscular administration, penethamate

25 hydroiodide is absorbed from the site of injection and on entering the blood partially dissociates by hydrolysis into benzylpenicillin and diethylaminoethanol. At blood pH (7.2), an

equilibrium is established wherein 91.8% of the active drug is present in its hydrolysed form (benzylpenicillin) with the remainder being penethamate. The equilibrium is maintained by re-association of benzylpenicillin and diethylaminoethanol. Peak serum levels (measured as dissociated penicillin G) are rapidly reached, 3.76 hours after injection (Friton et al., Journal 5 of Veterinary Pharmacology and Therapeutics, 2003. vol. 26, Issue S1 (A-23 (0091)): p. 100-101).

The un-disassociated form of penethamate easily passes over the blood-milk barrier due to the pH gradient present between milk (pH 6.6-6.8) and plasma (pH 7.2) and its weakly basic state ($pK_a = 8.4$). The lipophilic nature of penethamate further facilitates its passage across 10 the lipo-proteinic blood-milk barrier. Penethamate starts to dissociate as it passes over the blood-milk barrier and this continues during diffusion of the drug throughout the udder, releasing benzylpenicillin. The benzylpenicillin is rapidly ionised in the udder ($pK_a = 2.8$), as a result of the lower pH of milk, trapping the active within the udder in increasing concentrations.

15 Current PNT products available on the market for intramuscular (IM) or sub-cutaneous (SC) injection, are in the form of a powder for reconstitution into solution or suspension with sterile water at the time of use (e.g. Mamyzin™ Boehringer Ingelheim or Penethaject™ Bayer Animal Health). Typical dosages of these products include three daily doses of 5g of PNT, or one dose of 10g followed by a 5g dose the next day.

20 The use of an aqueous vehicle allows for the PNT to dissolve quickly at the injection site and thus be rapidly absorbed. Especially desirable properties for treating mastitis during the lactation period include rapid absorption of the active, effective therapeutic action, and a short withhold period. The ability of penethamate to cross the blood/milk barrier and concentrate in the udder, provides effective therapeutic action and a sufficiently short 25 withhold period.

Despite their effectiveness as therapeutic actives, a disadvantage of currently available compositions as injections containing penicillin or PNT is their limited shelf-life (often only 2-

3 days) once reconstituted into an aqueous solution from a powder. This is inconvenient to the user, who must reconstitute the powder into a liquid form, such as by drawing out a sterile aqueous vehicle from one vial, dispense the liquid into a second vial containing the powder and mix until a solution or homogeneous suspension is formed. Once the product is

5 reconstituted it must be used within the limited period of stability, or it must be discarded.

Currently, there is no ready to use IM/SC injectable formulation of PNT to treat bovine mastitis or other diseases available on the market. A significant problem in developing a ready to use injectable formulation is the lack of storage stability of PNT in aqueous vehicles. The use of non-aqueous vehicles has been avoided for PNT formulations, since oil vehicles

10 in particular typically provide a much slower release of the active after IM/SC injection. A slow release of the active would extend the withhold period for lactating cows, which is especially undesirable and would likely prevent commercialisation of the product.

The published patent document US 4,446,144 mentions that PNT can be used in suspensions or solutions in suitable vehicle which can be made of an aqueous or oily base.

15 Non-aqueous vehicles are indicated as providing better stability. The formulations described in US 4,446,144 are for parenteral use, e.g. injections given as an aqueous solution or suspension. No mention is made of the desirability of obtaining fast-release of the active or bioequivalence with aqueous formulations.

There has been limited research in formulating a PNT composition for a IM/SC injection

20 using a non-aqueous vehicle. Edwards, S.J. (1964), The Veterinary Record, Vol. 78, No. 17, 583-5, documents a study of penicillin levels in milk following intramuscular injection.

The prior art products requiring reconstitution of PNT into an aqueous vehicle provide a compromise, with the advantage of the rapid absorption of the active when administered, with the disadvantage of the limited storage stability of the reconstituted composition.

25 There has been a long felt need for a PNT composition that is ready to use, has good storage stability, and rapid release of the active.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge 5 the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents form part of the common general knowledge in the art, in New Zealand or in any other country.

Throughout this specification, the word "comprise", or variations thereof such as "comprises" 10 or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

15 DISCLOSURE OF INVENTION

According to one aspect of the present invention there is provided a composition, including penethamate (PNT) or a pharmaceutical equivalent thereof; and at least one oily vehicle.

Preferably the viscosity of the composition is below 3000 mPas at a temperature of 20°C 20 and shear rate of 1/s measured with a cup cylinder method.

Oily vehicle here also includes non-aqueous vehicles which are only partially soluble with water – for example a preferred oily vehicle is triacetin. Triacetin has a viscosity of approximately 23 mPas at 20°C and a density of 1.16g/cm³.

Preferably the penethamate (PNT) or pharmaceutical equivalent thereof comprises 55% w/v

or less of the composition.

Preferably the composition includes at least one anticaking agent.

According to a further aspect of the present invention there is provided a composition, including

- 5 penethamate (PNT) or pharmaceutical equivalent thereof;

at least one oily vehicle; and

at least one non-thickening anticaking agent.

According to a further aspect of the present invention there is provided a composition, the composition including:

- 10 penethamate (PNT) or pharmaceutical equivalent thereof;

and a low viscosity oily vehicle.

Preferably the composition comprises a suspension of penethamate (PNT) or pharmaceutical equivalent thereof.

According to a further aspect of the present invention there is provided a composition, the

- 15 composition including

penethamate (PNT) or pharmaceutical equivalent thereof;

and at least one oily vehicle having a viscosity range between 2.0 to 100.0 mPa at 25°C.

The inventors have formulated PNT compositions that overcome many of the disadvantages of the prior art PNT composition.

- 20 The present invention presents a ready to use injectable composition of PNT that can provide similar bioavailability or bioequivalence to aqueous based injectable.

The compositions of the invention advantageously avoid hydrolysis of the active by using an oily vehicle. Initial studies have shown substantially no degradation of the PNT active or other stability issues even after storage over 180 days at 30°C.

This is a clear advantage over prior art formulations such as Penethaject™, which has a

- 5 shelf life of approximately only two days after reconstitution and storage at 25°C (or just one week when stored at 2-8°C). Users may be able to store the composition of the present invention in a ready-to-use liquid preparation, for example in a pre-prepared syringe. In large dairy farms, and particularly during the milking season, this invention presents a significant contribution to the art over other compositions currently used in the industry.
- 10 Various oil based compositions are previously known in the art for delivering antibiotics as an injectable liquid. Oil is used to avoid hydrolysis (i.e. degradation and hence poor stability) of the active, that may result from the use of an aqueous base. However, the use of oils have been used to provide a sustained release profile of the active over a relatively long period of time.
- 15 The present invention was surprisingly provides a fast release of the PNT and a short withholding period (WHP). These advantageous features are both provided by the aqueous based composition Penethaject™ for treatment/prevention of mastitis during the lactation period. This is contrary to what was expected.

Previously available oily injectable formulations have a significantly longer sustained release

- 20 profiles.

Therefore, the present invention may have particular application to treatment or prevention of conditions such as mastitis in lactating animals, where a fast release, short persistency and short WHP is desired.

Additionally, the use of the present invention was also surprisingly found to have a

- 25 bioavailability profile similar and potentially better than the prior art aqueous based formulations such as Penethaject™. The prior art has focused on provision of PNT

formulations in aqueous based systems because of the required bioavailability. While it has also been expected that an oily formulation will result in slower release and longer withhold times.

Therefore, the inventors have identified a solution to address the current problem of poor

5 stability and shelf life of PNT compositions suitable or SC/IM injection for treatment of mastitis. The present invention also provides means for improving the WHP and bioavailability characteristics compared with other veterinary compositions typically used for treatment or prevention of mastitis during an animal's lactation period.

Further while oily vehicles have been used with benzyl penicillin (Penicillin G) and/or

10 procaine penicillin (see Chinese Patent No. 1517090) tests by the inventors have shown these to be chemically unstable on storage. Thus the long term stability of PNT with triacetin (or other oily vehicles) is highly unexpected.

In particular, the inventors trials have determined that when procaine penicillin is substituted for the penethamate in the preferred formulation of the present invention, the resulting 15 composition is chemically unstable. After one month in accelerated stability the components of the composition appeared to degrade rapidly with a resulting colour change from an off-white to a dark yellow with dark brown sludge forming. The degradation components appeared to include a gas, resulting in the container swelling from the resultant pressure. Accordingly, the formulations described in CN 1517090 are not known to be storage stable.

20 The use of triacetin provided a storage stable formulation, both chemically and physically. The triacetin alone advantageously prevented the caking issues of the other oily vehicle formulations. The addition of the surface active agents further improved the re-suspendability of the composition on storage.

The withhold time was found to be equivalent to that of a known but unstable product called

25 Mamyzin™, and it was bio-equivalent.

Throughout this specification, the term penethamate (PNT) should be taken as meaning the

diethylaminoethyl ester prodrug of benzyl penicillin (BP). It is BP that is the antibacterial active agent relied on in the present invention, and as well known in the industry.

It has been found that an oily vehicle gives the desired results with regards to a WHP and bioavailability profile comparable to currently available aqueous based penethamate

5 compositions.

The type and amount of oily vehicle may be selected from any appropriate oily vehicle and amount thereof which provides the desired drug release and/or withhold period.

Preferably, the ratio of PNT to oily vehicle is between 1:1 to 1:4 w/v.

However, the composition of the present invention may also be used to treat other bacterial

10 infections similar to those treated with Penethaject™ such as metritis, respiratory infections and footrot in cattle and horses.

Preferably the oily vehicle is ethyl oleate, medium chain triglycerides or triacetin.

During initial trials, ethyl oleate was exemplified to show the advantageous characteristics of the present invention, namely increased stability, and maintained (if not improved) WHP and

15 bioavailability compared to Penethaject™.

A prior art document CN 101822637 discloses the use of ethyl oleate as the carrier for β -lactam antibiotics. The compositions include 2-5% of active, with suspending agents 2%, thickeners 0.1-3% and preservatives such as benzyl alcohol. The suspending agents include aluminium stearate, which would increase the viscosity, and the thickening agents are said to

20 include Tween® 80.

The present invention is distinguished from this document in that it uses specifically penethamate hydroiodide, which as an ester of a β -lactam may be expected to have different properties and carrier requirements. The amount of antibiotic is significantly different, since the minimum of 20% PNT in the current formulations poses different

25 problems to the 2-5% of the prior art document. When using ethyl oleate as the vehicle, the

present invention does not use a thickener as a suspending agent, rather it relies on the surface active agents to provide re-suspendability of the composition, rather than attempting to prevent settling of the suspension. When using ethyl oleate the present invention was found to have long-term stability of up to three months.

- 5 Yet, subsequent trials have shown that triacetin is a far better vehicle, being suitable for injection and less likely to cause caking of the suspended PNT.

However, it should be appreciated that a number of different oily vehicles may be used, yet still provide the requirements which lead to the beneficial results as described herein.

Clearly, a person skilled in the art would be able to recognise, without undue

- 10 experimentation, other oily vehicles may be used as a substitute for ethyl oleate medium chain triglycerides, triacetin or a combination of at least two oily vehicles.

Other examples of vehicles considered appropriate to use include soybean oil, cotton seed oil, corn oil, sunflower oil, peanut oil, sesame oil, and paraffin oil. Other oily vehicles are considered within the scope of the invention.

- 15 Preferably, the concentration of PNT in the composition is between 15% - 55% w/v.

Most preferably, the concentration of PNT in the composition is between 20% to 35% w/v.

The inventors identified these preferred amounts of PNT in the composition may be sufficient to provide about 5 g of active to the animal in a given dosage. In previous studies, this amount of PNT which was identified to provide a therapeutic effect similar to PenethajectTM.

- 20 Preferably, the particle diameter d_{50} of the PNT is between 1 – 100 microns.

Most preferably, the particle diameter d_{50} of the PNT is between 8 - 30 microns with 95% < 50 microns.

This is the approximate particle size of the PNT in the currently available PenethajectTM

formulation. This feature may be important to help provide or improve bioavailability profiles of the composition.

In a particularly preferred embodiment, the composition includes at least one excipient which prevents caking.

5 Surprisingly, the inclusion of an additional excipient such as certain surfactants can dramatically improve the physical stability of PNT non-aqueous suspensions. For example, polysorbate 80 works particularly well in a preferred embodiment.

Typically, suspensions are physically stabilized by adding a standard anti-caking agent or thickener such as colloidal silicon dioxide or aluminium stearate. While colloidal silicon

10 dioxide should not be administered parenterally ('Handbook of Pharmaceutical Excipients', Raymond C Rowe, Fourth Edition (2003), pages 161 – 164), aluminium stearate thickens the suspension which slows down the drug release from the vehicle. These agents do not deliver a practical solution to physically stabilise PNT suspensions.

It was found that triacetin is a vehicle that dramatically improves physical stability of PNT

15 suspension but at the same time delivers satisfying chemical stability. This is surprising given that triacetin is partially soluble with water, (while still being considered an oily vehicle) which would be expected to lead to hydrolysis of the active.

On the other hand non-oily vehicles such as water and propylene glycol deliver good physical stability of PNT suspensions, but such PNT suspensions are only chemically stable

20 for a few days (e.g. water based Mamyzin™) or less than 2 months during storage for propylene glycol. Again, this does not deliver a practical solution for a ready to use PNT composition with acceptable shelf life.

Surprisingly, it was also found that oily vehicles such as ethyl oleate or medium chain triglycerides in combination with specific excipients improves the physical stability of PNT

25 suspension without compromising on chemical stability.

The hydrophobic-lipophilic balance (HLB) value is an indication of the solubility of the surfactant. The lower the HLB value the more lipophilic or oil soluble the surfactant is. The higher the HLB value the more water soluble or hydrophilic the surfactant is. The HLB values are used for nonionic surfactants only.

- 5 In formulations that do not use triacetin as an oily vehicle (e.g ethyl oleate instead) then surface active agents such as emulsifiers and surfactants are essential to prevent caking (mass agglomeration of particles).

Span® 80 (sorbitan mono-oleate) with a relatively low HLB value of 4.3, is soluble in the oily vehicle, but provided little improvement of the physical stability of the PNT suspension.

- 10 Tween® 80 (polysorbate 80) with a relatively high HLB value of 15.0, is practically insoluble in the oily vehicle, and significantly improved the physical stability of the PNT suspension. The insoluble surfactant PEG12Oleate (HLB of 13.7) in oily vehicle also significantly improved the physical stability.

Surprisingly, it was found that certain types of lecithin can dramatically improve the physical

- 15 stability of PNT suspensions while other types of lecithin do not. For instance a practically insoluble (at room temperature) hydrogenated soybean lecithin in oily vehicle has a positive impact on the physical stability of PNT suspensions but a solubilized soybean lecithin in an oily vehicle does not.

It was also found that adding Span® 80 to an oily vehicle consisting of a medium chain

- 20 triglyceride and a hydrogenated soybean lecithin does not negatively impact on the physical stability of PNT suspensions, but can increase the bioavailability of the PNT.

However, it should be appreciated that a number of different surfactants may be substituted for the above ones, yet still provide a physically stable PNT suspension, without compromising on chemical stability which lead to the beneficial results as described herein.

- 25 A person skilled in the art would be able to readily ascertain the ability of any particular surfactant to stabilise a suspension of PNT.

Further, the inclusion of a surfactant did not deleteriously affect the beneficial stability nor the short WHP of the composition.

Contrary to expectations and the typical properties of drug release from an oily vehicle, it was found that the PNT release from an oily vehicle according to the present invention could

- 5 be as quick, or even quicker as the control aqueous based formulations.

This fast release profile from an oily vehicle may be important as it allows quick absorption of PNT, and hence provides a fast therapeutic effect.

Preferably, with non-triacetin based formulations the surfactant is selected from a compound with a hydrophobic-lipophilic balance (HLB) of approximately 7-16.

- 10 More preferably, the surfactant has an HLB of above 12.

In preferred embodiments with triacetin as the oily vehicle, the surfactant is hydrogenated soybean lecithin. It should be noted that as lecithin contains ionic components, the HLB system cannot be properly applied.

This surfactant was shown to substantially improve the release of PNT from the oily base

- 15 after administration (see Example 3 in the Best Modes section). Given the results exemplified herein, one skilled in the art would expect that a similar surfactant or surfactants to hydrogenated soybean lecithin for instance falling within the HLB range of 7-16, would also provide a similar beneficial effect to the current composition.

However Tween® 80 has shown significantly better results when with triacetin as the vehicle.

- 20 It works well as an anti-caking compound plus does not have thickening properties.

In preferred embodiments at least one preservative is included. For example, methyl paraben and propyl paraben or benzyl alcohol may be used as preservatives.

Benzyl alcohol is preferred with triacetin based formulations as it mixes well with triacetin, maintains its activity in oily vehicles, as well as being suitable for injections.

For the usefulness of the product, the preservative is important since it allows the ready-to-use composition to be used on multiple occasions. A study was performed to test the stability and sterility of the present invention product after removal of a dose from the container:

In this study the penethamate suspension (in accordance with the preferred formulation),

5 packed in 100 mL clear PET vials stored at room temperature were broached, using 10 mL sterile disposable syringe with 16G hypodermic needle, each week for four weeks. On each occasion an appropriate amount of sample was removed and after day 28, the left over sample was analysed for physical, chemical and microbial characteristics. The results show that the penethamate suspension, remains physically and chemically stable after repeated

10 broaching over a period of 28 days at room temperature.

Further the sterility of the samples was not compromised by repeated broaching after in-use stability trial period of 28 days.

Method of treatment

According to another aspect of the present invention there is provided a method of treating

15 an animal with a composition substantially as described herein for the treatment or prevention of a microbial infection, wherein the method includes intramuscular or subcutaneous injection of the composition to the animal in need thereof.

Preferably, the microbial infection is pre-clinical or clinical mastitis.

Preferably, the method of treatment includes a dosage regime of 5 g PNT per day repeated

20 for approximately three days.

An alternative dosage regime may include delivery of 10 g PNT as a first dose on a first day, followed by a further dose of 5 g PNT on the second day. Such dosages are similar to that currently advised for Penethaject™.

According to another aspect of the present invention there is provided a use, in the

25 manufacture of a composition as substantially described herein, for treating or prevent a

microbial infection in an animal.

Method of Manufacture

According to a further aspect of the present invention there is provided a method of manufacturing the composition as substantially described above including the steps of:

- 5 a) Preparing an oily vehicle by either (i) providing an oil, or (ii) mixing an oil and surfactant(s) in a container to form a homogenous oil mixture;
- b) dispersing the active agent in the oily vehicle.

Optionally, at least one preservative is added to the oily vehicle in step a). Preferably, the oily vehicle of step a) is sterilized by filtration.

- 10 Preferably, step b) utilises high shear dispersion equipment.

An additional advantage of the composition's preferred low viscosity is beneficial to the manufacturing process.

Likewise, the lower viscosity allows for easier filling of containers, such as vials or syringes, following manufacturing of the composition.

- 15 Preferred the active agents are micronised. The use of micronized active agents can help prevent quick settling of the solids within the composition.

BRIEF DESCRIPTION OF DRAWINGS

Further aspects of the present invention will become apparent from the ensuing description which is given by way of example only and with reference to the accompanying drawings in

- 20 which:

Figure 1 Mean benzylpenicillin plasma and milk concentrations following intramuscular administration of MamyzinTM;

Figure 2 Benzylpenicillin milk concentrations following intramuscular administration of PNT from Edwards 1965;

Figure 3 Benzylpenicillin milk concentrations following intramuscular administration of different PNT compositions;

5 Figure 4 Benzylpenicillin plasma concentrations following intramuscular administration of different PNT compositions;

Figure 5 Benzylpenicillin milk concentrations following intramuscular administration of Mamyzin™ and composition OT11PNTRTU-d;

10 Figure 6 Inhibitory substance concentration in milk following intramuscular administration of compositions OT11PNTRTU-e, -f, and -g;

Figure 7 Inhibitory substance concentration in milk following intramuscular administration of compositions OT11PNTRTU-f, and -h;

Figure 8 Inhibitory substance concentration in milk following intramuscular administration of compositions OT11PNTRTU-i, and -j;

15 Figure 9 Benzylpenicillin milk concentration following intramuscular administration of Penethaject™ and compositions OT11PNTRTU-e, and -k;

Figure 10 Benzylpenicillin milk concentration following intramuscular administration of different dosage regimes of composition OT11PNTRTU-e;

20 Figure 11 Benzylpenicillin milk concentration in plasma following intramuscular administration of Penethaject™ and compositions OT11PNTRTU-e, and -k;

Figure 12 Relative bioavailability of benzylpenicillin following intramuscular administration of compositions OT11PNTRTU-e, and -k in relation to Penethaject™;

Figure 13 Relative bioavailability of benzylpenicillin following intramuscular administration of compositions OT11PNTRTU-I, -d, -a and -b in relation to PenethajectTM;

Figure 14 Recovery percentage of PNT from compositions OT11PNTRTU-I, -d and -n during stability trials;

5 Figure 15 Recovery percentage of PNT from compositions OT11PNTRTU-o, and -p during stability trials.

Figure 16 Depletion of benzyl/penicillin in milk from Example 6.

BEST MODES FOR CARRYING OUT THE INVENTION

A number of formulations exemplifying the present invention are given below.

Example 1: Exemplification formulations according to the present invention, all percentages in example 1 are % w/w.

5

OT11PNTRTU-a		
	g	%
Penthemate Hydriodide	5.0	31.3
Span® 80	0.080	0.50
Propyl parabens	0.003	0.02
Methyl parabens	0.013	0.08
Ethyl Oleate	3.271	20.45
Sesame oil	7.63	47.71
total	16.00	100.0%

OT11PNTRTU-b		
	g	%
Penthemate Hydriodide	5.0	31.3
Span® 80	0.080	0.50
Propyl parabens	0.003	0.02
Methyl parabens	0.013	0.08
Sesame Oil	10.90	68.15
total	16.00	100.0%

OT11PNTRTU-c		
	g	%
Penthemate Hydriodide	5.0	31.3
PEG12Oleate	0.048	0.30
Propyl parabens	0.003	0.02
Methyl parabens	0.013	0.08
Aerosil® R972	0.160	1.00
Miglyol® 812	10.78	67.35
total	16.00	100.0%

OT11PNTRTU-d		
	g	%
Penthamate Hydriodide	5.0	33.3
PEG12Oleate	0.075	0.50
Propyl parabens	0.003	0.02
Methyl parabens	0.012	0.08
Ethyl Oleate	9.91	66.07
total	15.00	100.0%

OTPNTRTU-e		
	g	%
Penethamate Hydriodide	5.0	35.0
Tween® 80	0.022	0.15
Ethyloleate	9.28	64.89
total	14.30	100.0%

OTPNTRTU-f		
	g	%
Penethamate Hydriodide	5.0	34.96
Span® 80	0.022	0.15
Ethyloleate	9.28	64.89
total	14.30	100.0%

OTPNTRTU-g		
	g	%
Penethamate Hydriodide	5.0	35.0
Tween® 80	0.022	0.15
Span® 80	0.022	0.15
Ethyloleate	9.26	64.74
total	14.30	100.0%

OTPNTRTU-h		
	g	%
Penethamate Hydriodide	5.0	35.0
Span® 80	1.430	10.00
Ethyloleate	7.87	55.03
total	14.30	100.0%

OTPNTRTU-i		
	g	%
Penethamate Hydriodide	5.0	35.0
Tween® 80	0.143	1.00
Ethyloleate	9.16	64.0
total	14.30	100.0%

OTPNTRTU-j		
	g	%
Penethamate Hydriodide	5.0	35.0
Tween® 80	1.430	10.0
Ethyloleate	7.87	55.0
total	14.30	100.0%

OTPNTRTU-k		
	g	%
Penethamate Hydriodide	5.0	35.0
Ethyloleate	9.30	65.0
total	14.30	100.0%

OT11PNTRTU-i		
	g	%
Penthemate Hydriodide	5.0	22.7
Tween® 80	0.110	0.50
Propyl parabens	0.004	0.02
Methyl parabens	0.018	0.08
Propylene Glycol	16.87	76.67
total	22.00	100.0%

OT11PNTRTU-m		
	g	%
Penthemate Hydriodide	5.0	31.3
Span® 80	0.080	0.50
Propyl parabens	0.003	0.02
Methyl parabens	0.013	0.08
Miglyol® 840	10.90	68.15
total	16.00	100.0%

OT11PNTRTU-n		
	g	%
Penthemate Hydriodide	5.0	22.7
Tween® 80	0.077	0.35
Span® 80	0.033	0.15
Propyl parabens	0.004	0.02
Methyl parabens	0.018	0.08
Ethyl Oleate	16.87	76.67
total	22.00	100.0%

OT12PNTRTU-q		
	g	%
Penthemate Hydriodide	5	20.5
Benzyl Alcohol	0.2443	1
Tween® 80	1.222	5.00
Span® 80	0.024	0.10
Triacetin	17.94	73.43
total	24.43	100%

OT12PNTRTU-r		
	g	%
Penthemate Hydriodide	5.0	23.9
Benzyl alcohol	0.20	1.0
Tween® 80	0.073	0.35
Span® 80	0.031	0.15
Lipoid® 90H	0.076	0.36
Miglyol® 812	15.50	74.23
total	20.88	100%

OT12PNTRTU-s		
	g	%
Penthemate Hydriodide	5.0	24.4
Benzyl alcohol	0.2	1.0
Tween® 80	0.072	0.35
Span® 80	0.031	0.15
Ethyl Oleate	12.1	59.0
Triacetin	3.1	15.1
total	20.503	100.0%

OT12PNTRTU-t		
	g	%
Penthemate Hydriodide	3.4	34.00
Benzyl alcohol	0.1	1.00
Polysorbate 80	0.01	0.10
Lecithin (phospholipon® 90 H)	0.036	0.36
Triacetin	6.454	64.54
total	10.0	100.0%

Example 2: Exemplification of short withhold period

Surprisingly it was found that by controlling the viscosity of the compositions OT11PNTRTU-a and -b, the drug release from the compositions at the site of injection is fast enough in

5 order to achieve a WHP in milk of equal or smaller than 60 hours, or considering confidence intervals as requested from regulatory authorities up to 72 hours, as shown in Figure 3.

Both compositions were given as a 10g PNT dose. WHP (withhold period) is here defined as when the concentration curve cuts the MRL line followed by the next multiple of 12. For instance a calculated WHP of 52 hours would provide a registered WHP of 60 hours. This

10 also includes that some registration authorities around the world require a consideration of a confidence interval for determination of WHP. These results are especially surprising if one compares the results shown in Figure 3 with the data from Edwards, S.J. (1964), The Veterinary Record, Vol. 78, No. 17, 583-5, as shown in Figure 2 for 5 MioIU (equivalent to 5g PNT) oil based formulation.

15 The Edwards data would suggest a much longer WHP than 84 hours with only half the dose (5g PNT). The composition of the oily vehicle used by Edwards was not disclosed in the published document, however the findings of Edwards is consistent with the general knowledge of oil based vehicles, that the release of active is expected to be slower.

The absorption rate of PNT is a crucial component for a treatment of mastitis in lactating

20 cows. Oil based compositions as injectables are generally regarded as slow release dosage forms. In general, the absorption rate of injectable compositions is fast for aqueous solutions

containing a drug with hydrophilic properties and slow for oily solutions containing a drug with lipophilic properties. A prediction of the absorption rate of a drug from an oily suspension is usually based on the properties of the drug.

The composition of OT11PNTRTU-c with a much higher viscosity compared to

5 OT11PNTRTU-a and -b, has a WHP longer than 96 hours. Table 1 lists the viscosities of the compositions tested. Figure 4 shows that OT11PNTRTU-c is more slowly absorbed into blood after a 10 g PNT dose compared to all other compositions in Figure 4 and has still elevated concentrations at 36 hours and 48 hours of BP in plasma after the last treatment, leading to a longer WHP compared to the other compositions.

10 Table 1: Viscosity of test compositions -a, -b, -c, and PenethajectTM

Composition	Viscosity mPas at 20°C, Shear rate 1/s
Penethaject TM	280
OT11PNTRTU-a	380
OT11PNTRTU-b	770
OT11PNTRTU-c	3270

It is expected that the PNT compositions disclosed in US 4,446,144 as examples 51 to 53 would also have a similarly slow release and long withhold period, due to the substantial amount of thickener, in the form of 12-hydroxystearin and aluminium monostearate.

Further it was found for a 10g PNT dose that the WHP in milk of the oil based composition

15 OT11PNTRTU-d is almost identical to the aqueous based MamyzinTM composition (after reconstitution), as shown in Figure 5. This is surprising considering that oil based formulations are generally regarded as slow release formulations, and particularly with regard to the lipophilic nature of PNT.

It was also found that with increasing surfactant concentration the WHP can be further

20 decreased. As shown in Figure 7 a comparison is made between OT11PNTRTU-f that

contains 0.15% Span® 80, and OT11PNTRTU-h that contains 10% Span® 80. Similarly Figure 8 compares OT11PNTRTU-I that contains 1.00% Tween® 80 with OT11PNTRTU-j that contains 10% Tween® 80. It is clear that the increase in surfactant corresponds to a decrease in the withhold period.

- 5 Figure 9 shows the BP concentration in milk for compositions of the present invention over time after three injections (according to a proposed dosage regime), with a comparison between OT11PNTRTU-e, OT11PNTRTU-k and Penethaject™. As can be seen, the calculated WHP following ACVM guidelines of the tested compositions is similar (approximately 42-46 hours) to Penethaject™ as currently available on the market.
- 10 Surprisingly it was found that this short withhold period is possible for ethyl oleate based composition without any surfactant (OT11PNTRTU-k).

Further it was surprisingly found that the calculated WHP for the dosage regimes of 10g followed by 5g PNT the next day, and 3x5g PNT daily doses are both below 48 hours and therefore would have 48 hour WHP following the calculations of New Zealand ACVM guidelines, as shown in Figure 10. This is surprisingly advantageous to the aqueous suspension Mamyzin™, which following a 10g + 5g PNT dosage regime has a 60 hour WHP.

Example 3: Exemplification of bioavailability

- Figure 3 shows the BP concentration in milk for various test compositions, Mamyzin™ and Penethaject™ following a 10g dose of PNT. Again, it can be seen that the compositions
- 20 OT11PNTRTU-a and -b substantially align with the release profile of Mamyzin™.

Bioequivalence is here defined as when the difference of the area under the curve between the reference product (aqueous based PNT composition) and test product (oil based PNT composition) both given as the same dose (relative bioavailability) is not more than 20% (bioequivalent =1; limits from 0.9 to 1.1) (ACVM REGISTRATION STANDARD AND

25 GUIDELINE FOR THERAPEUTIC EQUIVALENCE OF TRADE NAME PRODUCTS).

Figure 4 shows the BP concentration in plasma for the compositions OT11PNTRTU-a, -b and -c, as compared to Penethaject™ following a 10g dose of PNT. Again, it can be seen that the compositions OT11PNTRTU-a and -b substantially align with the release profile of Penethaject™.

5 The relative bioavailability for the BP plasma results are shown in Table 2. The compositions OT11PNTRTU-a and b meet the requirements of are bioequivalence or even higher bioavailability as defined above, whereas the high viscosity formulation OT11PNTRTU-c shows no bioequivalency.

Table 2: Relative bioavailability of test compositions -a, -b, and -c

Composition	Relative bioavailability $AUC_{24h, \text{ oil}}/AUC_{24h, \text{ aq}}$
OT11PNTRTU-a	0.93
OT11PNTRTU-b	1.11
OT11PNTRTU-c	0.78

10 The AUC_{24h} of the oil based PNT compositions investigated by Edwards was significantly lower than the aqueous based PNT compositions at equivalent doses, as shown in Table 3. The viscous formulation OT11PNTRTU-c is comparable with the relative bioavailability results of the 5 MioIU oil formulation in Edwards. Although the Edwards data is in milk and Figure 4 shows data in plasma, it is still appropriate to compare the relative bioavailabilities

15 since there is a direct correlation between plasma and milk BP concentrations in respect to the area under the curve, as shown in Figure 1.

Table 3: Relative bioavailability of Edwards compositions

Composition	PNT dose Mio IU	AUC_{24h} IU/mL*h	Relative bioavailability $AUC_{24h, \text{ oil}}/AUC_{24h, \text{ aq}}$
Oil base	1	4.0	0.68
Aqueous base	1	5.9	

Oil base	2	5.9	0.86
Aqueous base	2	6.9	
Oil base	5	18.2	0.77
Aqueous base	5	23.7	

Furthermore it was found that different surfactants with different HLB values (Span® 80 and Tween® 80) or combinations thereof lead to similar BP concentrations in milk (figure 6).

It was surprisingly found that when used for a dosage regime of three daily doses of 5g PNT, the oil based compositions OT11PNTRTU-e and -k have almost the same BP concentrations

5 in milk as the aqueous solution based (when reconstituted) Penethaject™, as shown in Figure 9.

A further surprising result is that the mean maximum concentration c_{max} of BP in plasma after the last treatment of three daily doses of 5g PNT for the oil based compositions OT11PNTRTU-e and -k were larger compared to Penethaject™, as shown in Figure 11.

10 Further it was found that the relative bioavailability of the oil based compositions OT11PNTRTU-e and -k were larger than 1.0, which means that these oil based compositions have a higher bioavailability over 24 hours and overall than Penethaject™, as shown in Figure 12.

15 The relative bioavailabilities in plasma of the oil based compositions OT11PNTRTU-I, -d, -a, and -b following a 10g dose of PNT are shown in figure 13. The results demonstrate that the compositions are at least bioequivalent, or have or even higher bioavailability of Penethaject™.

20 It was very surprisingly to discover that that the bioavailabilities of the oily compositions are higher, or at least meet the lower limit of bioavailability to achieve bioequivalence, compared to an aqueous based PNT composition. Consequently, this also will have a positive impact on BP milk concentrations due to the direct correlation between blood and milk as shown in

Figure 1. This may allow a reduction in the PNT dose whilst still achieving the same bioavailability and efficacy. A reduction in the PNT dose may also lead to a shorter withhold period. Example 4: Exemplification of storage stability characteristics

It was found that PNT is not chemically stable in all non-aqueous formulations despite the
 5 assertion made in the patent document US 4,446,144. Figure 14 shows that PNT in propylene glycol (OT11PNTRTU-I) degrades about 30% w/w after around two months, whereas PNT is chemically stable for about six months in the formulations OT11PNTRTU-d and -m, at a temperature of 40°C and uncontrolled humidity conditions.

It was further surprisingly found that OT11PNTRTU-d and -n are chemically stable for at
 10 least three months at 40°C and 75 %rH even though PNT can hydrolyse in presence of water/moisture. The PNT recovery after two months is shown in Table 4.

Table 4: Stability of PNT in compositions OT11PNTRTU-d and -n

Composition	PNT recovery %
OT11PNTRTU-d	100.5
OT11PNTRTU-n	100.2

Glass containers typically deliver the best moisture protection. While PNT powder is stable in PET vials, it is surprising that PNT non-aqueous suspensions are also stable since the
 15 diffusion coefficient in liquids are significantly higher compared to powders.

It is evident that the present invention shows very good storage chemical stability of PNT over an extended period of time.

Example 5: Exemplification of physical stable non-aqueous PNT suspensions

For the treatment of bovine mastitis the PNT concentration in an injectable composition
 20 could be 20% w/v or more. While such suspensions of PNT and oil without any further excipients may appear physically stable in controlled laboratory conditions or during storage, under typical transporting and handling the suspensions can show caking behaviour.

Initial testing of the sedimentation and/or caking characteristics was performed by subjecting samples to real-world transportation conditions. More controlled and convenient testing was performed using purpose built mechanical agitation apparatus. The controlled laboratory experiments were found to be consistent with the results from the real-world conditions.

- 5 It has been found that for some compositions the suspended PNT particles settle on the bottom of the vial or other container, forming a sediment. While it is commonplace for suspensions to settle and form a sediment, to be useful in a practical product such a sediment should be relatively easily re-suspendable, and caking should be avoided. The prior art document US 4,446,144 suggests that vegetable oils should deliver the highest
- 10 degree of stability. However PNT suspensions in oil vehicles such as sesame oil, such as OT11PNTRTU-b are not re-suspendable by shaking within a practical timeframe.

The time taken to re-suspend caked compositions was over 30 minutes in some cases.

Preferably the time taken to re-suspend the settled particles of a composition is in the order of a few minutes at the most. Most preferably if re-suspension is necessary, it should take

- 15 less than 1 minute.

Accordingly, it is desirable to include an anti-caking agent in the composition of the invention.

To avoid long withhold periods and poor bioavailability, the formulations of the invention use anti-caking agents that do not substantially increase the viscosity of the composition. Without limitation, such non-thickening anti-caking agents include solvents, surfactants and

- 20 emulsifiers. These agents may have a minor effect on the viscosity of the composition, but it is not significant compared the anti-caking agents colloidal silica and aluminium stearate which are known to be thickeners.

Surfactants such as Tween® 80 (polysorbate 80) and PEG12Oleate have been found to provide a more stable suspension, that is more easily re-dispersed.

- 25 Surprisingly, it was found that by using a vehicle which has low water solubility such as

triacetin, the physical stability of the PNT suspension is dramatically improved during storage and transport. In one trial the composition 100 mL of the PNT suspension OT12PNTRTU-q was contained in a 100 mL PET vial.

A second surprising example is given with OT12PNTRTU-r which is based on a medium

5 chain triglyceride vehicle. The addition of a hydrogenated soybean lecithin to the PNT suspension significantly improved the physical stability.

A preferred composition is OT12PNTRTU-s, which includes triacetin as a vehicle and anti-caking agent, and Tween® 80 as an anti-caking agent, along with benzyl alcohol which has also been found to be beneficial to the stability and re-suspendability.

10 Example 6:

The preferred formulation is given below:

Ingredient	Quantity (g/L)	Function
Penethamate Hydriodide (micronized)	333.3 g	Active Ingredient
Benzyl Alcohol	10.0 g	Preservative
Polysorbate 80 (Tween® 80)	1.0 g	Surfactant
Phospholipon® H90 (lecithin)	3.6 g	Emulsifier/Dispersing Agent
Triacetin	q.s to 1L	Vehicle

In the final formulation, the amount of penethamate (33.33%) is selected to match the concentration of penethamate in the aqueous based pioneer product Mamyzin™ (when reconstituted). The concentration is high relative to other injectables, since penethamate

15 requires a reasonably high dose, while there is a practical minimum to the volume of liquid that can be injected into an animal at one site. This high proportion of suspended solid exacerbates the caking problem, since a large lump of solid cake is more difficult to resuspend than a small amount.

The amount of benzyl alcohol is a standard amount when used as preservative.

The quantities of polysorbate 80 and phospholipon H90 are standard amounts, and function to assist with the re-suspension of the active after storage. The triacetin alone overcomes much of the caking problem, but the dispersion and re-suspension is still improved (i.e. faster to re-suspend) with the addition of these agents.

5 The surfactant (polysorbate 80) also improves the manufacturing process, since it helps the triacetin carrier 'wet' the active much faster, allowing for more rapid dispersion. Without surfactant the active initially tends to float on top of the liquid carrier, and requires a lot of mixing to disperse it through as a suspension. In a bulk manufacturing process this would be a significant inconvenience.

10

Further Data

The stability of the final formulation has not yet been completed, but it appears that the shelf-life at room temperature will be at least 12 months.

15

Stability Trial:

Stability Parameters

Inspection	Acceptance criteria
Description	An off-white homogenous suspension. May separate on standing. Resuspends on shaking.
Relative Density	1.000 - 1.500 g/mL @ 20°C
Penethamate Hydriodide	29.99 – 36.66 % w/v
Sterility	By Direct Inoculation Method (BP) No growth in Fluid Thioglycollate Medium after 14 days (minimum) incubation at 30 - 35°C No growth in Soybean Casein Digest Medium after 14 days (minimum) incubation at 20 - 25°C.

20

Stability data:**Batch No.: T1958****Packaging: 100mL clear PET vials**

Test	Storage conditions	Months			
		0	1	3	6
Description	25°C/ 60 % RH	Complies	-	Complies	Complies
An off-white homogenous suspension	30°C/ 65 % RH	Complies	-	Complies	Complies
	40°C/ 75 % RH	Complies	Complies	Complies	Complies
Relative Density	25°C/ 60 % RH	1.224	-	1.223	1.224
1.000 - 1.500 g/mL @ 20°C	30°C/ 65 % RH	1.224	-	1.227	1.223
	40°C/ 75 % RH	1.224	1.224	1.224	1.221
Penethamate Hydriodide	25°C/ 60 % RH	33.44	-	33.6	33.8
29.99 – 36.66 % w/v	30°C/ 65 % RH	33.44	-	34.8	33.5
	40°C/ 75 % RH	33.44	33.57	34.0	32.4
Sterility	25°C/ 60 % RH	pass	-	pass	pass
Direct Inoculation Method	30°C/ 65 % RH	pass	-	pass	pass
	40°C/ 75 % RH	pass	*	pass	pass

*test was not performed; - test not required

5 Withhold time

A study was performed to determine the withhold time of the preferred embodiment, namely

Example 6. The study used 22 cows in which 5 g (15 mL) of penethamate was injected

intramuscularly on three consecutive days. Milk samples were collected from all cows, twice daily, up to 120 hours after the final injection. Milk from the milkings at 12, 36, 48, 60 and 72

hours were analysed for penicillin residues. The results shown in Figure 16 show that the

benzylpenicillin residue in the milk drops below the MRL (maximum residue limit) of 0.004

mg/kg at around 41 hours after the final injection. This would provide a withhold time, based

on twice daily milkings, of 48 hours.

This is equivalent to the 48 hour withhold time of the product Mamyzin™, which is an

aqueous reconstituted suspension.

Bioequivalence

A study was performed to determine the bioequivalence of Example 6 with Mamyzin™.

A 2X2 crossover study using 20 dairy heifers was performed. All heifers received a 15 mg/kg intramuscular dose of penethamate hydroiodide on Day 0 and on Day 14, as the formulation in Example 6 or 'Mamyzin™'. After a 14 day wash-out period, treatments were reversed.

5 Blood was collected pretreatment, then 1, 2, 3, 4, 5, 6, 9, 12, 18, 24 & 36 hrs after each treatment. Blood was centrifuged and plasma samples were frozen, then dispatched to the laboratory for benzylpenicillin analysis (LC/MS/MS assay). Assay results were used to calculate pharmacokinetic parameters, to investigate bioequivalence. According to the 'area under the curve' (AUC) of the drug concentrations in blood over time, the preferred treatment

10 is bioequivalent to Mamyzin™.

The preferred manufacturing process is as follows:

Formulation Process

- Add 56% of Triacetin to a suitably sized manufacturing vessel
- Add Benzyl Alcohol and Polysorbate 80 with mixing

15 • Filter the mixture through a sterile 0.2µm filter into sterile tank

- Heat to approximately 55°C, check temperature and maintain the temperature
- Add Phospholipon® H90, suspend and homogenise well. Check homogeneity
- Add penethamate hydroiodide (micronised, sterile), suspend and homogenise well.

Check homogeneity

20 • Cool suspension to approximately 25°C. Check temperature

- Make up to volume with Triacetin.
- Homogenise to homogeneous suspension, check homogeneity.

This formulation and process has produced a stable non-caking injectable product that works at least as well as conventional PNT treatments.

25 It has a viscosity of 77cps @ 20°C on Brookfield viscometer with a LV1 spindle at 30rpm.
The density is 1.224g/cm³.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope of the appended claims.

WHAT I CLAIM IS:

1. A ready-to-use (RTU) composition for intramuscular or subcutaneous injection, including penethamate hydroiodide at a concentration of between 15 – 55% w/v; at least one oily vehicle; and an anti-caking agent selected from the group consisting of triacetin, polysorbate 80, PEG12Oleate or lecithin; wherein the viscosity of the composition is below 3000 mPas at a temperature of 20°C and a shear rate of 1/s measured with a cup cylinder method.
2. A composition as claimed in claim 1 wherein the anti-caking agent includes polysorbate 80.
3. A composition as claimed in claim 1 wherein the anti-caking agent includes a lipid-insoluble lecithin.
4. A composition as claimed in claim 1 wherein the anti-caking agent includes triacetin.
5. A composition as claimed in claim 1 wherein the oily vehicle includes an oil selected from the group consisting of ethyl oleate, triacetin, caprylic/capric triglyceride, propylene glycol dicaprylate/dicaprate, and sesame oil.
6. A composition as claimed in any one of claims 1 to 5 wherein the concentration of penethamate (PNT) in the composition is between 20-35% w/v.
7. A composition as claimed in any one of claims 1 to 5 wherein the particle diameter d_{50} of the penethamate (PNT) is between 8 – 30 microns.
8. A composition as claimed in any one of claims 1 to 5 which further includes a preservative.

9. A composition as claimed in claim 8 wherein the preservative is benzyl alcohol.
10. A syringe containing a composition as claimed in any one of claims 1 to 5.
11. A syringe as claimed in claim 10 which includes 5g of penethamate.
12. A use of a composition as claimed in any one of claims 1 to 5 for the treatment or prevention of a microbial infection.
13. A use as claimed in claim 12 wherein the microbial infection is pre-clinical or clinical mastitis.
14. A use as claimed in claim 12 wherein the use includes a dosage regime of 5g penethamate per day repeated for approximately three days.
15. A method of manufacturing the composition as claimed in any one of claims 1 to 5 including the steps of:
 - a) preparing the oily vehicle by (i) mixing an oil and anti-caking agent in a container to form homogenous oil mixture; and
 - b) dispersing the penethamate hydroiodide in the oily vehicle.
16. A method of manufacturing as claimed in claim 15 wherein a preservative is added to the oily vehicle in step a).
17. A method of manufacturing as claimed in claim 15 wherein high shear dispersion equipment is used in step b).

FIGURE 1

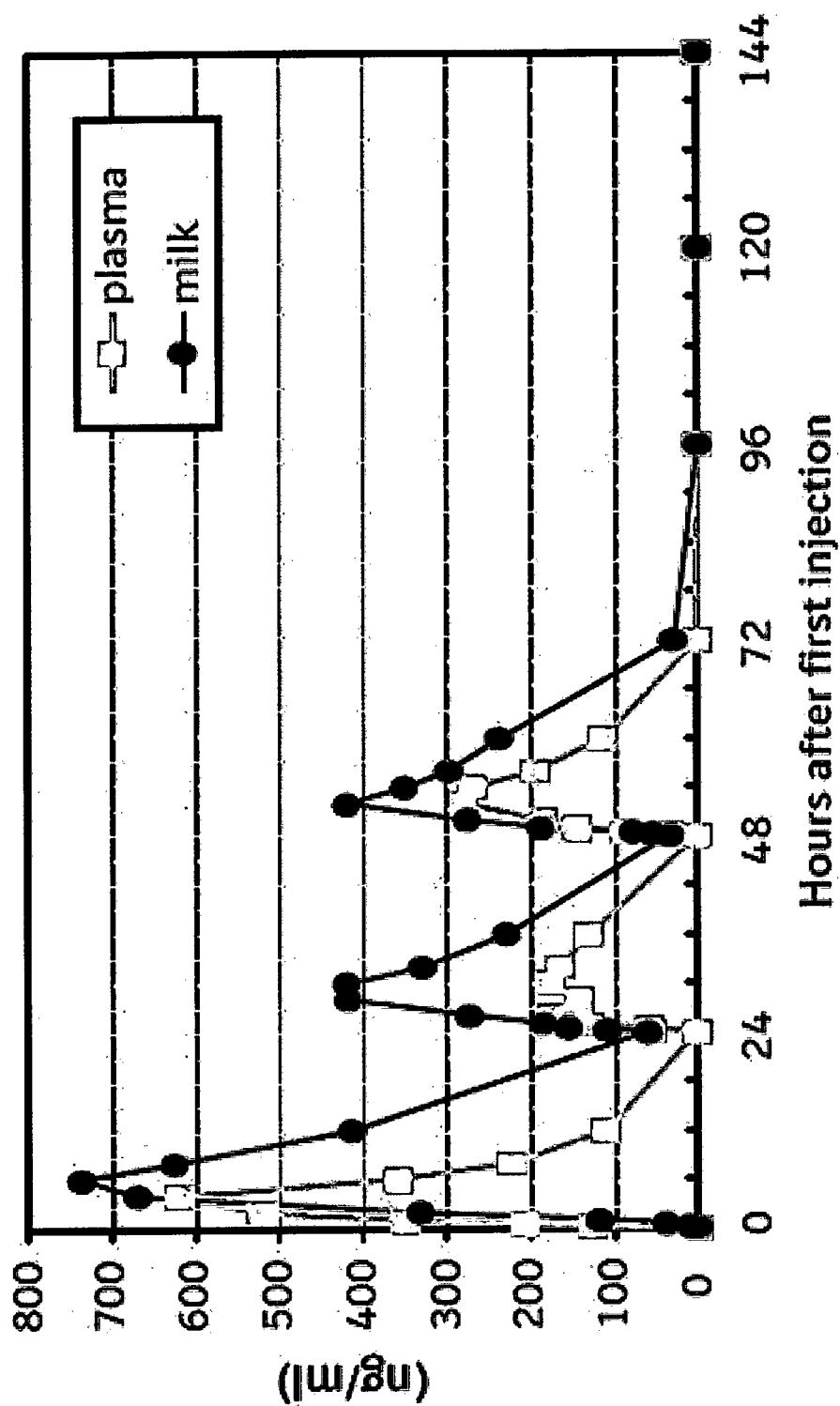


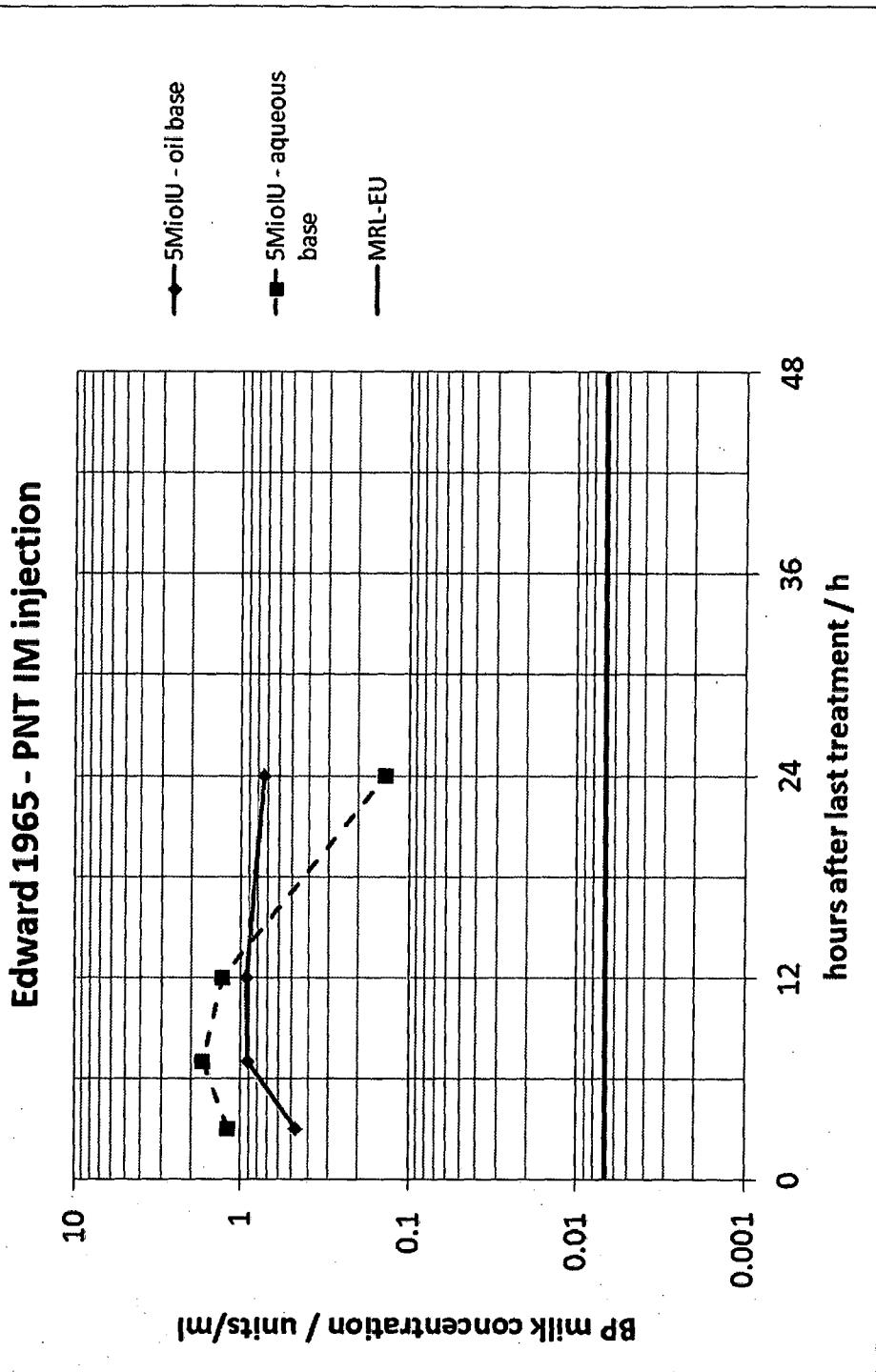
FIGURE 2

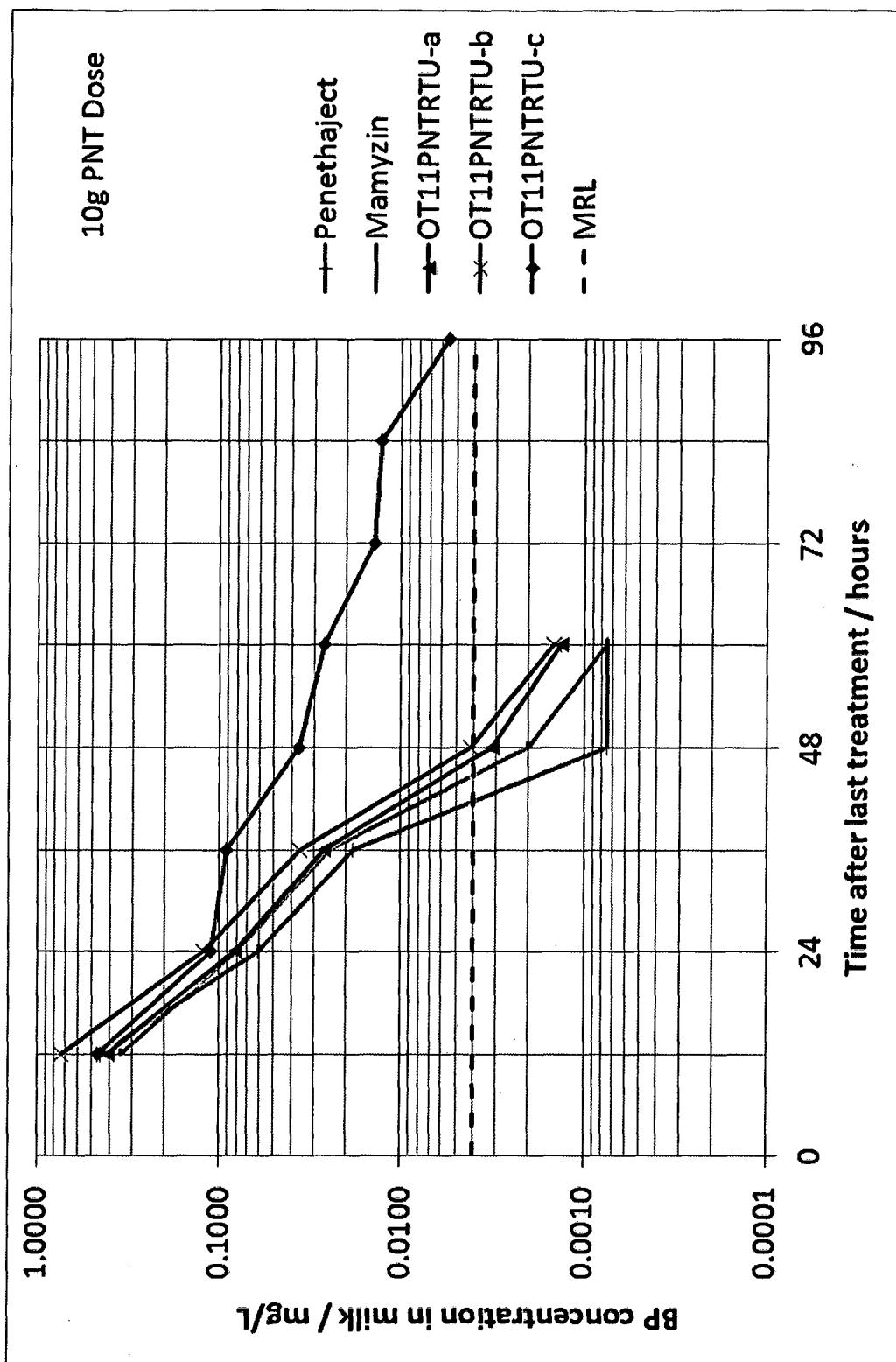
FIGURE 3

FIGURE 4

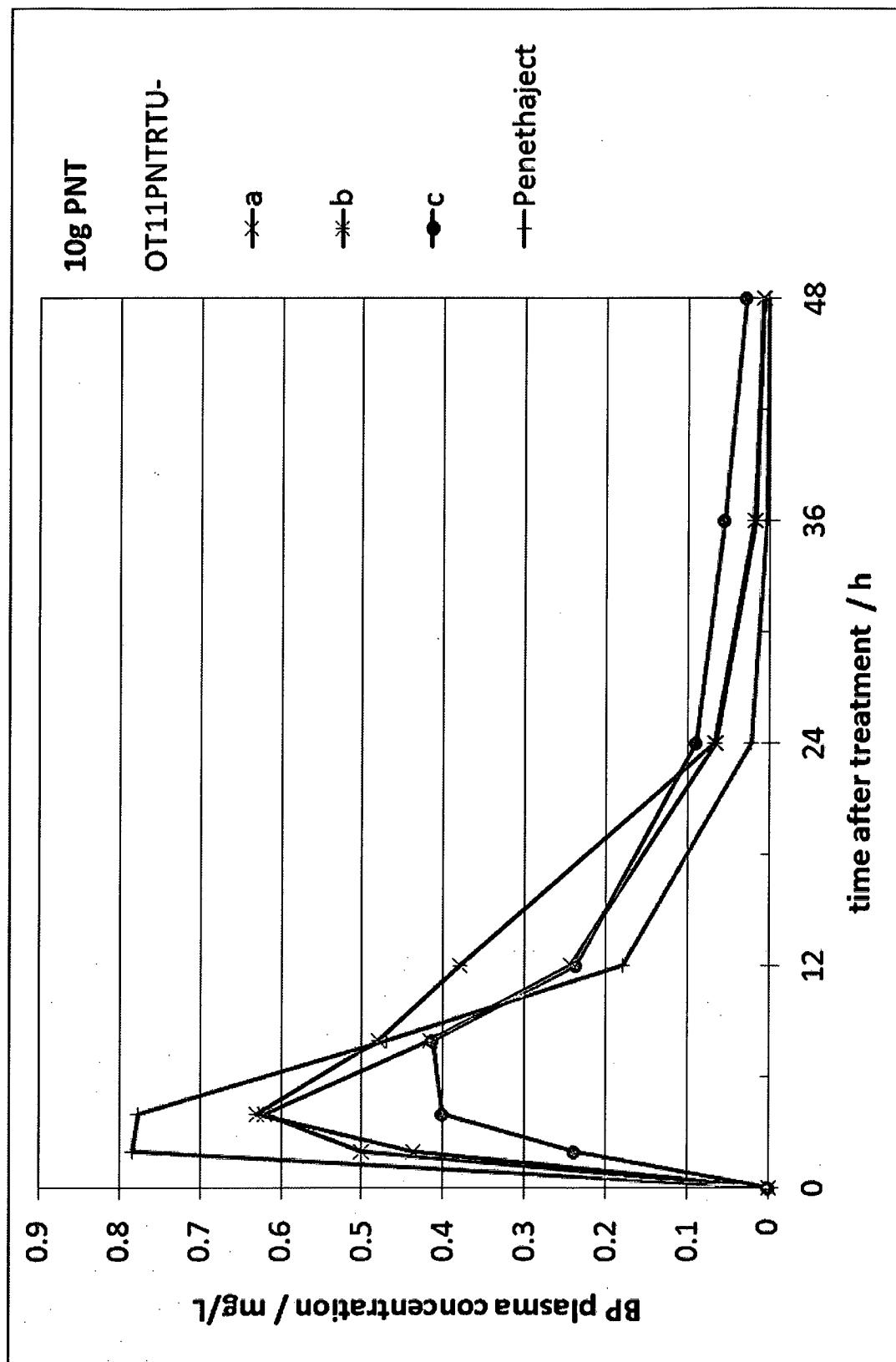


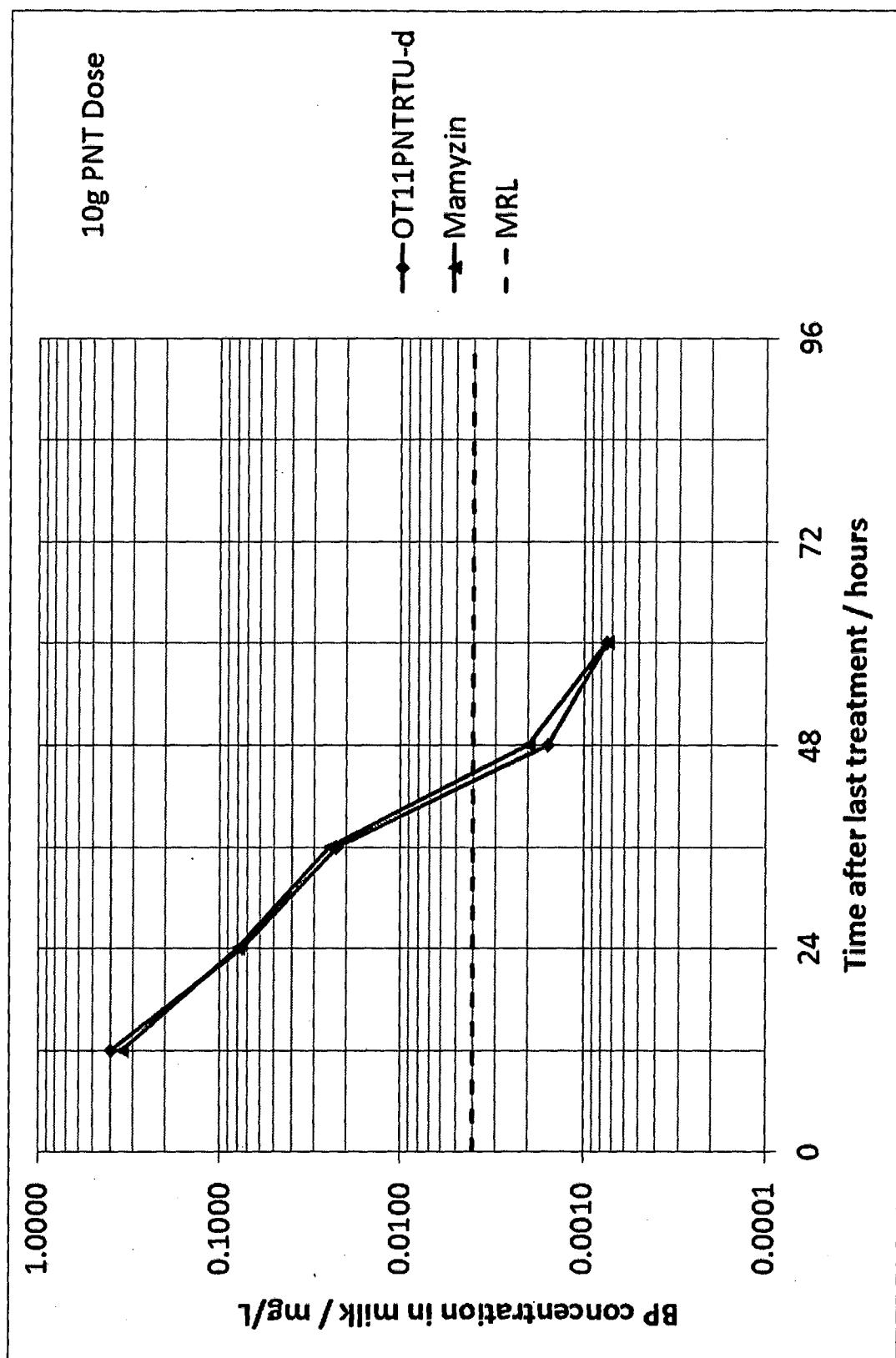
FIGURE 5

FIGURE 6

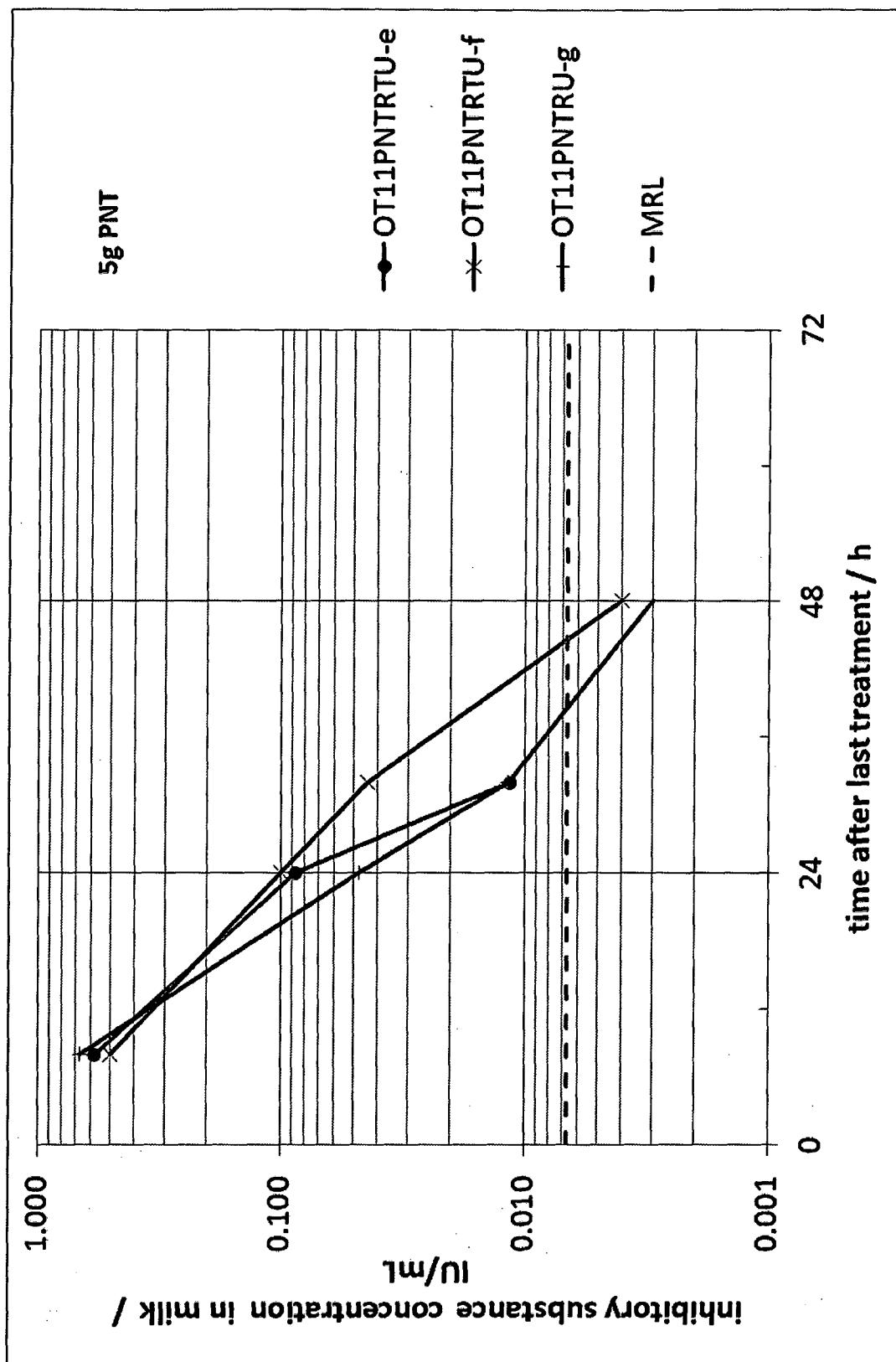


FIGURE 7

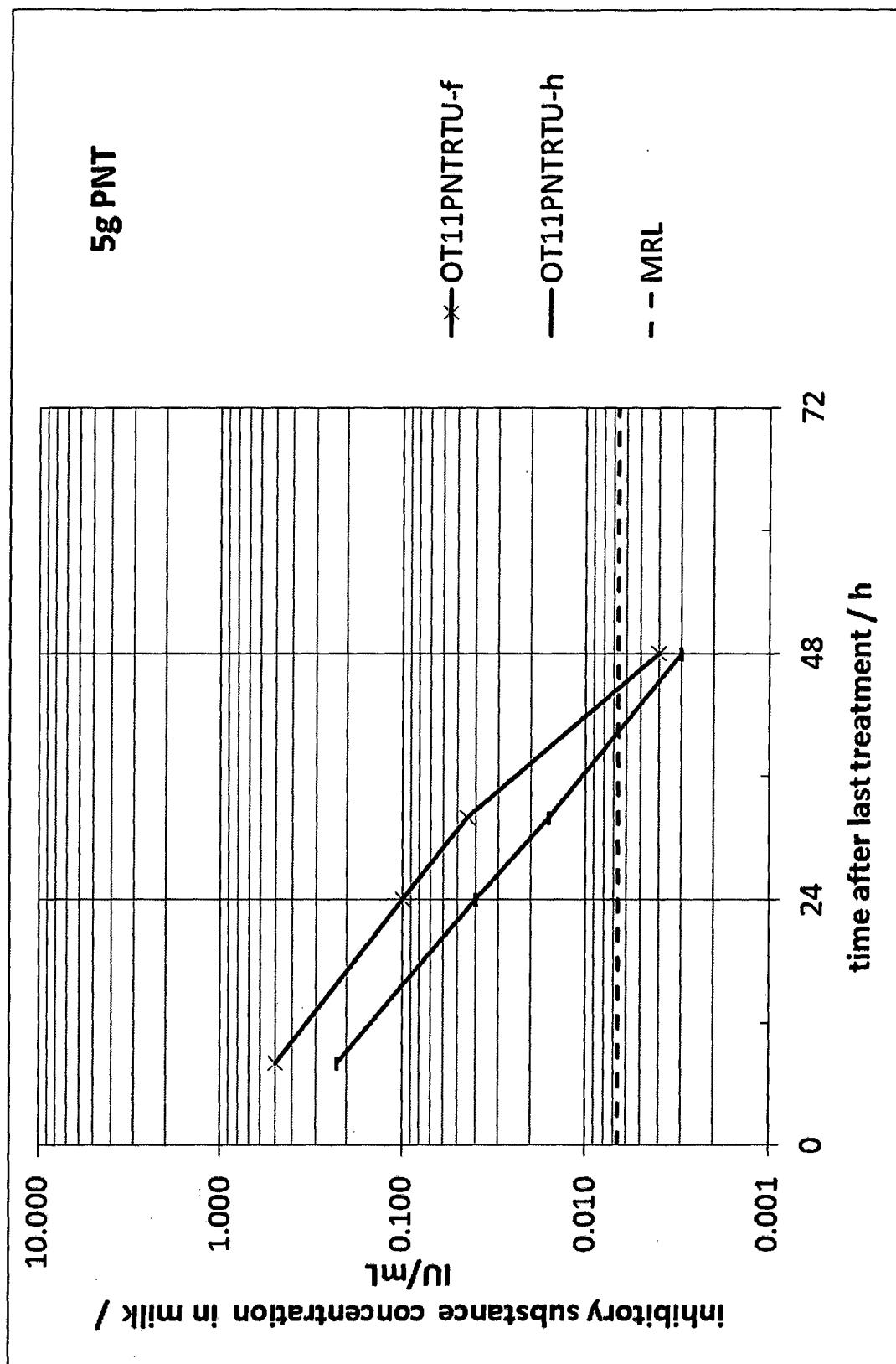


FIGURE 8

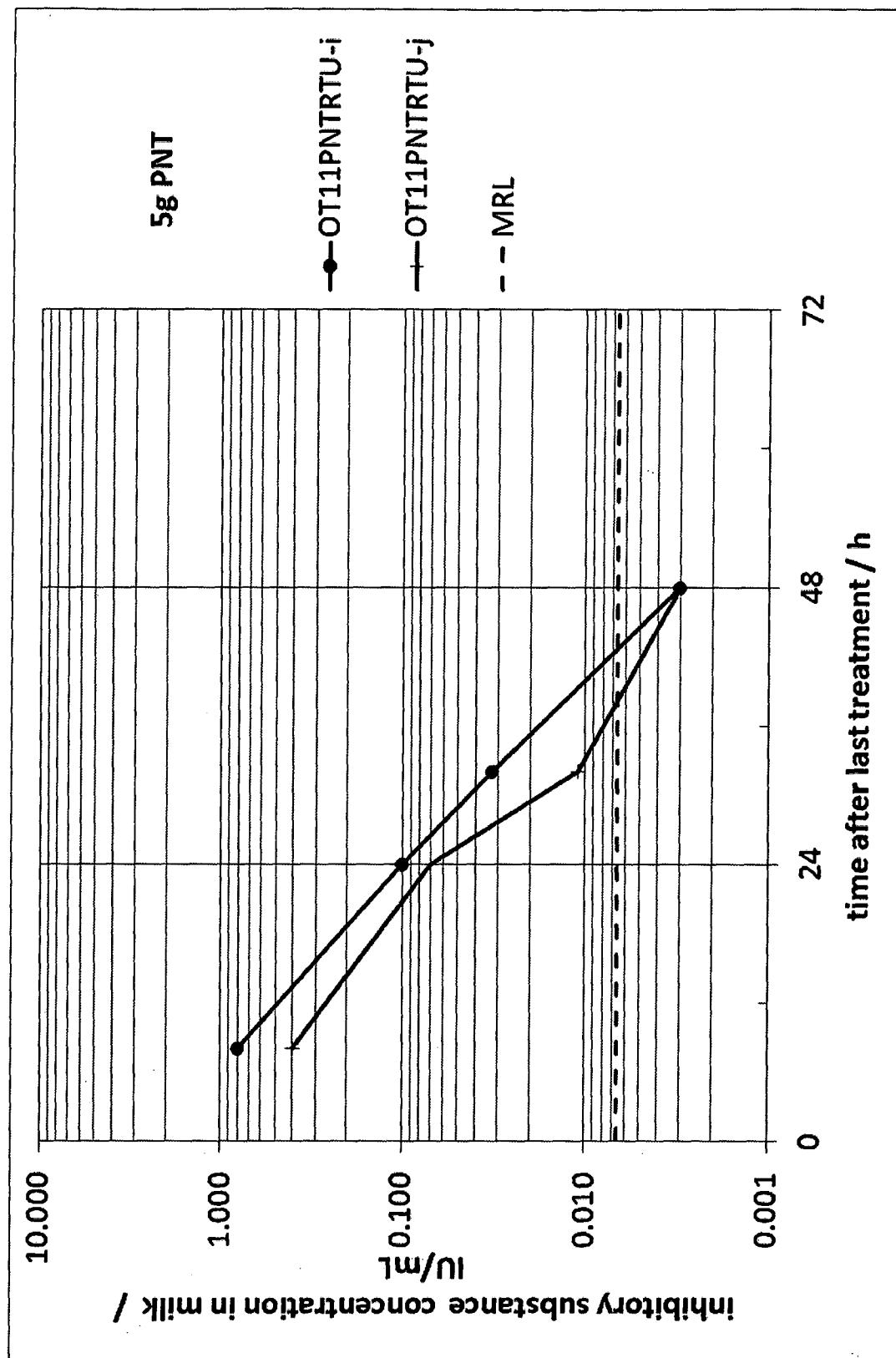


FIGURE 9

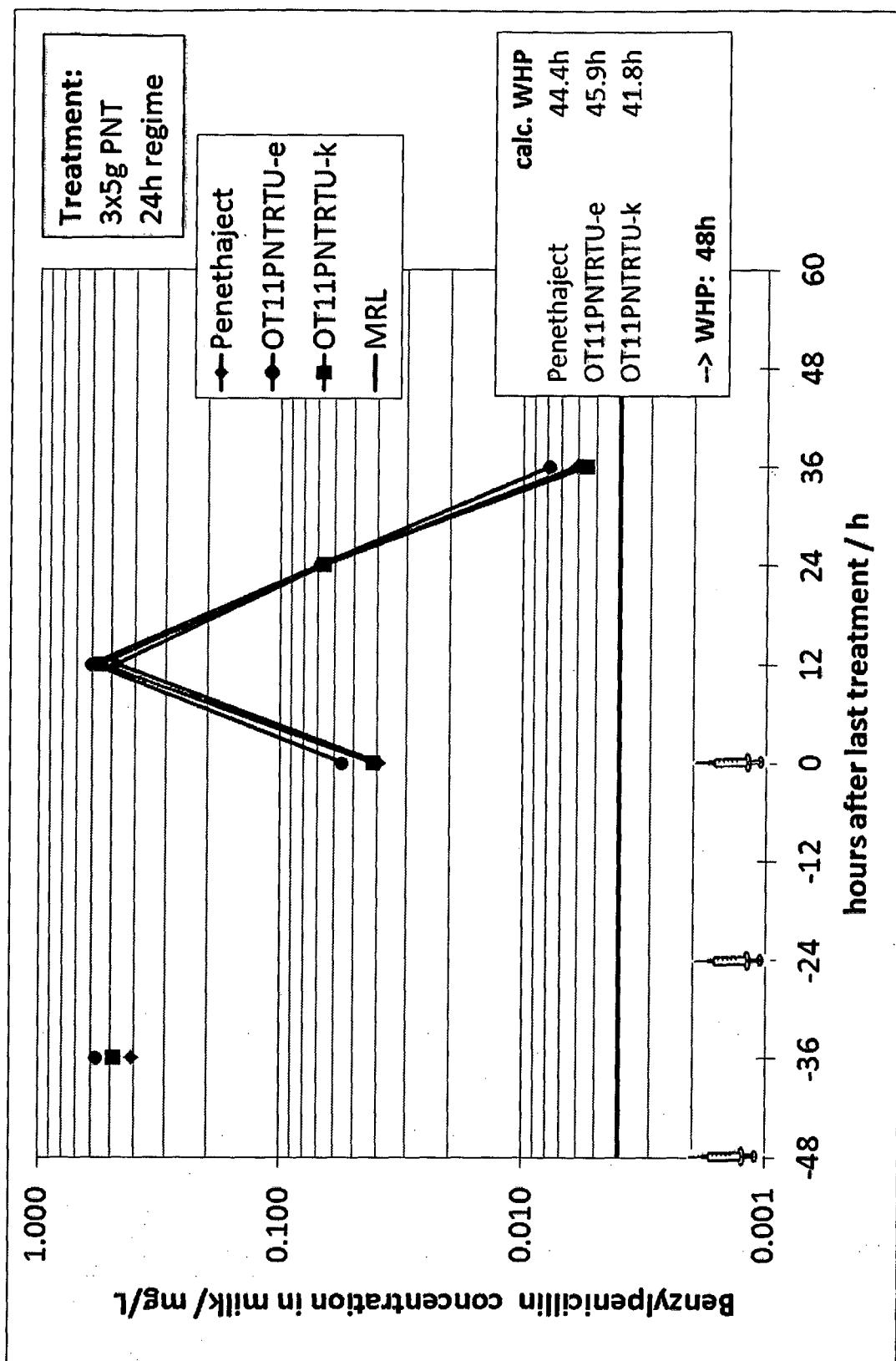


FIGURE 10

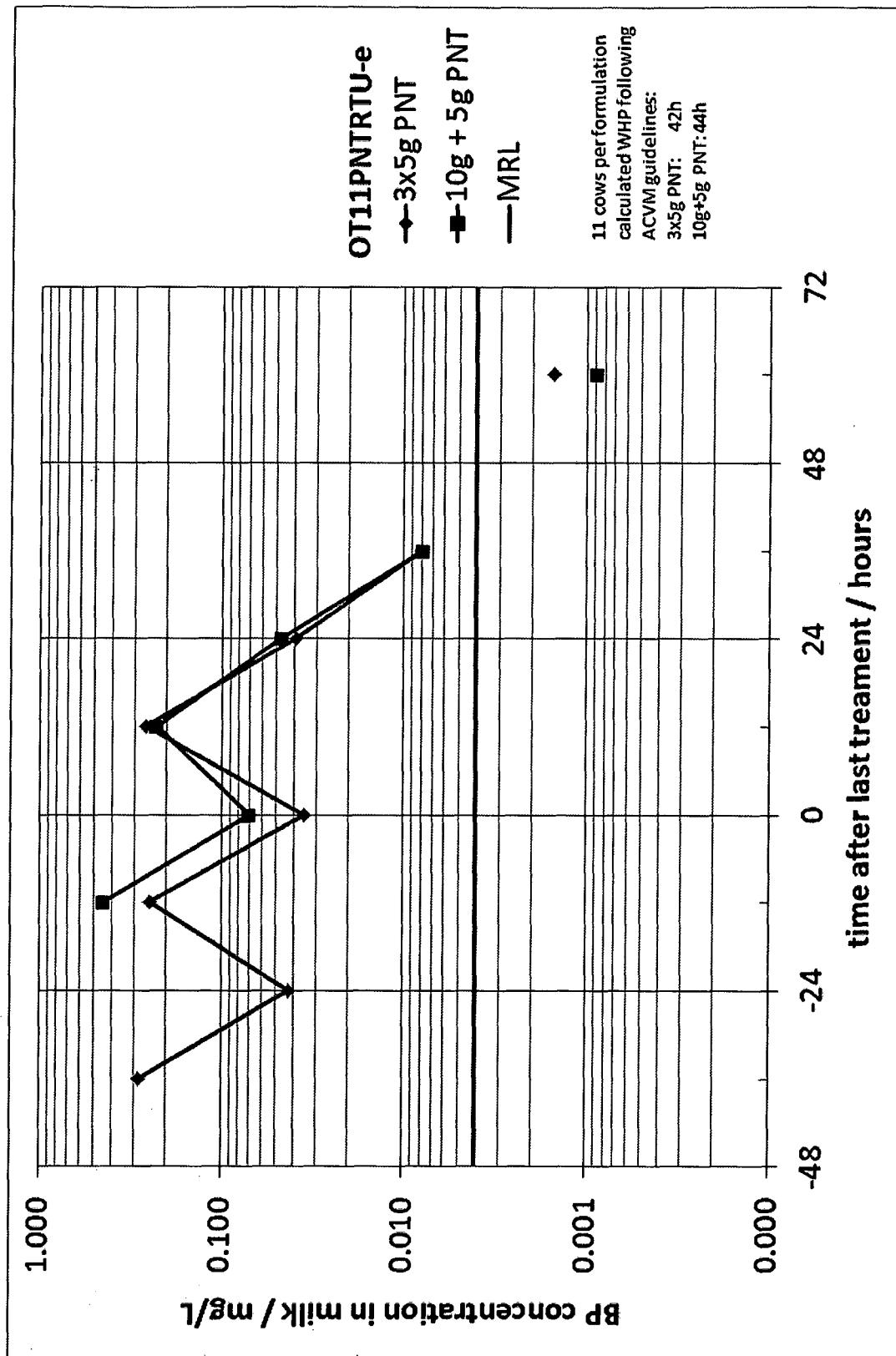


FIGURE 11

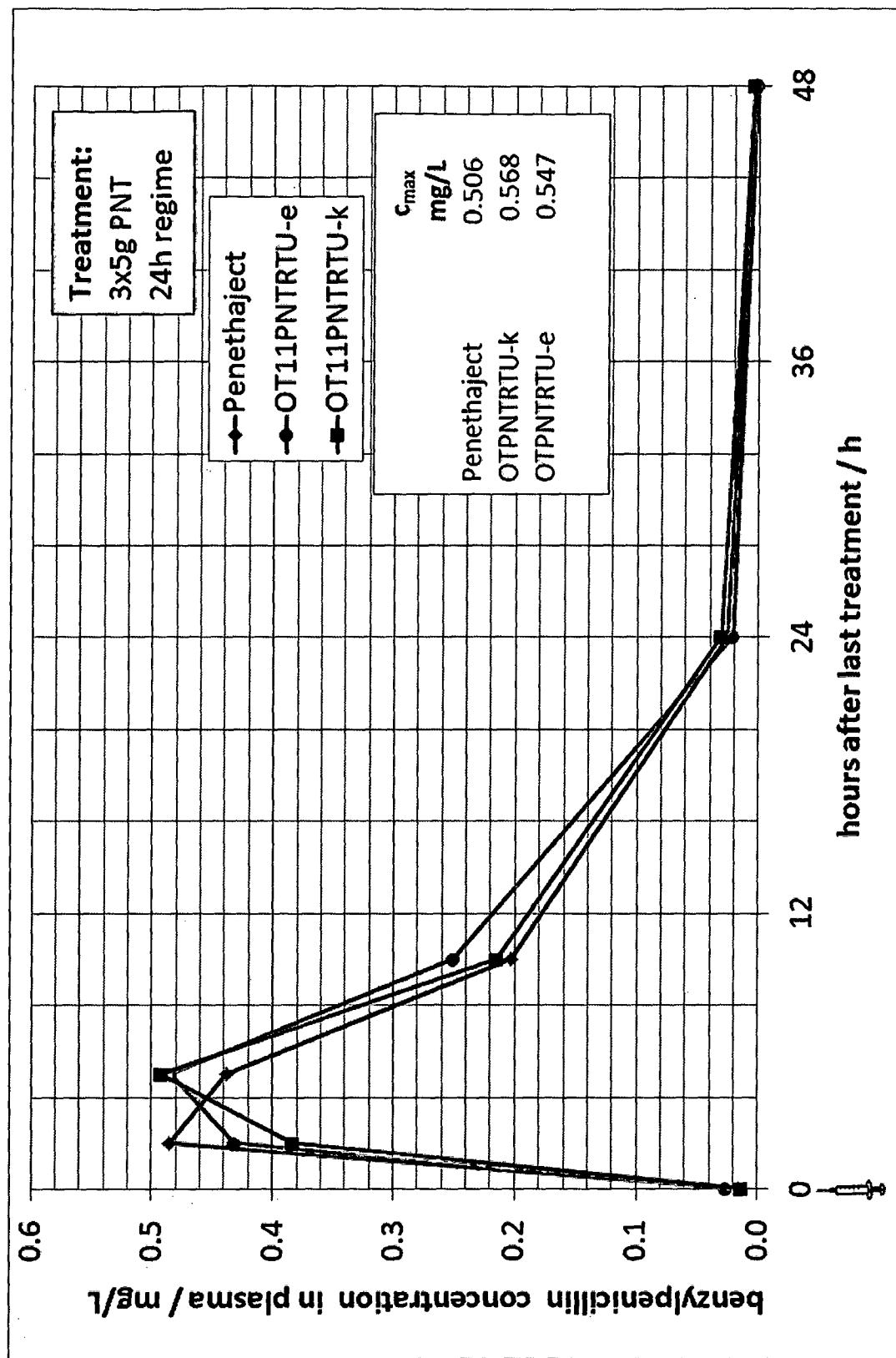


FIGURE 12

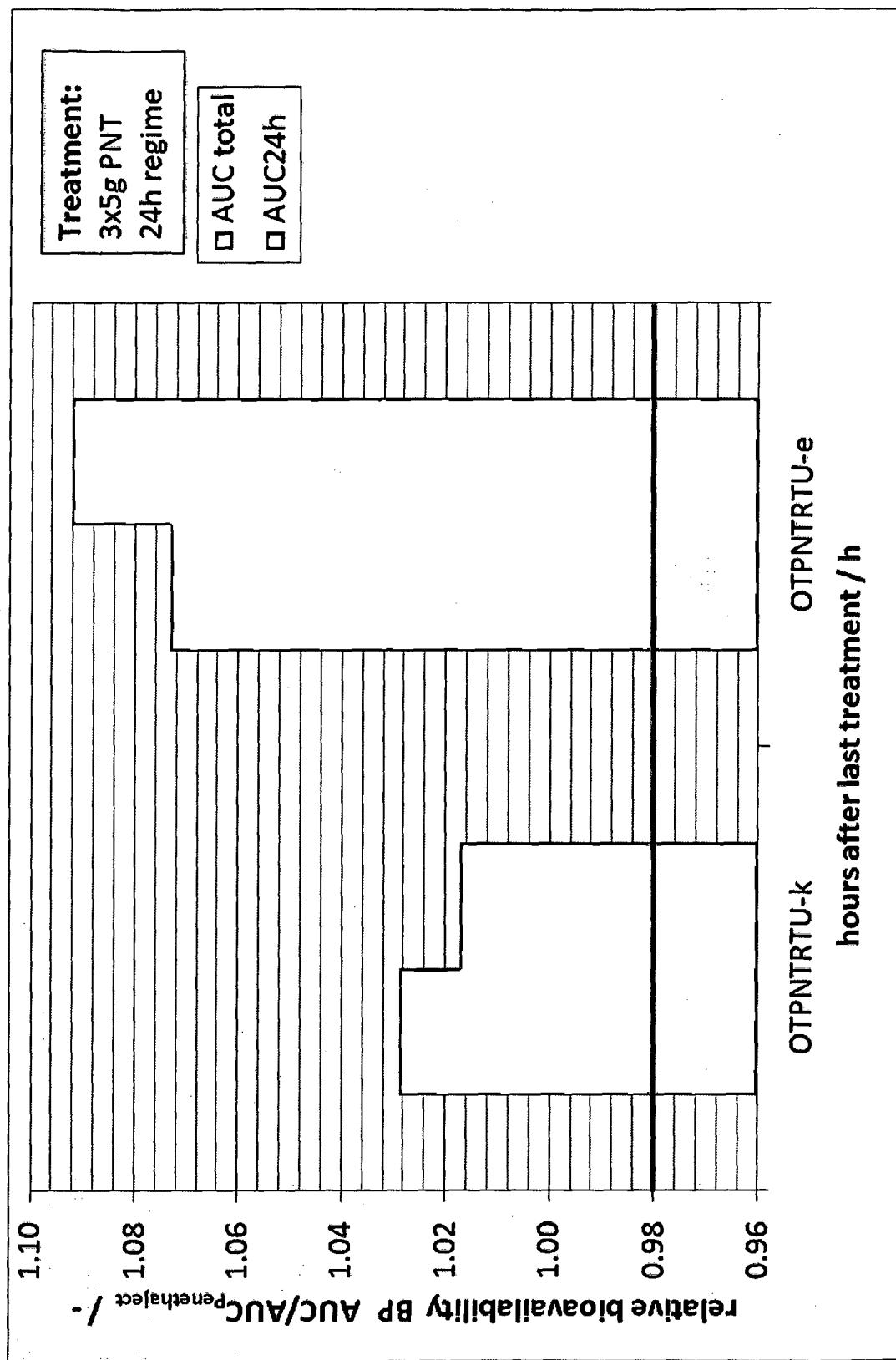


FIGURE 13

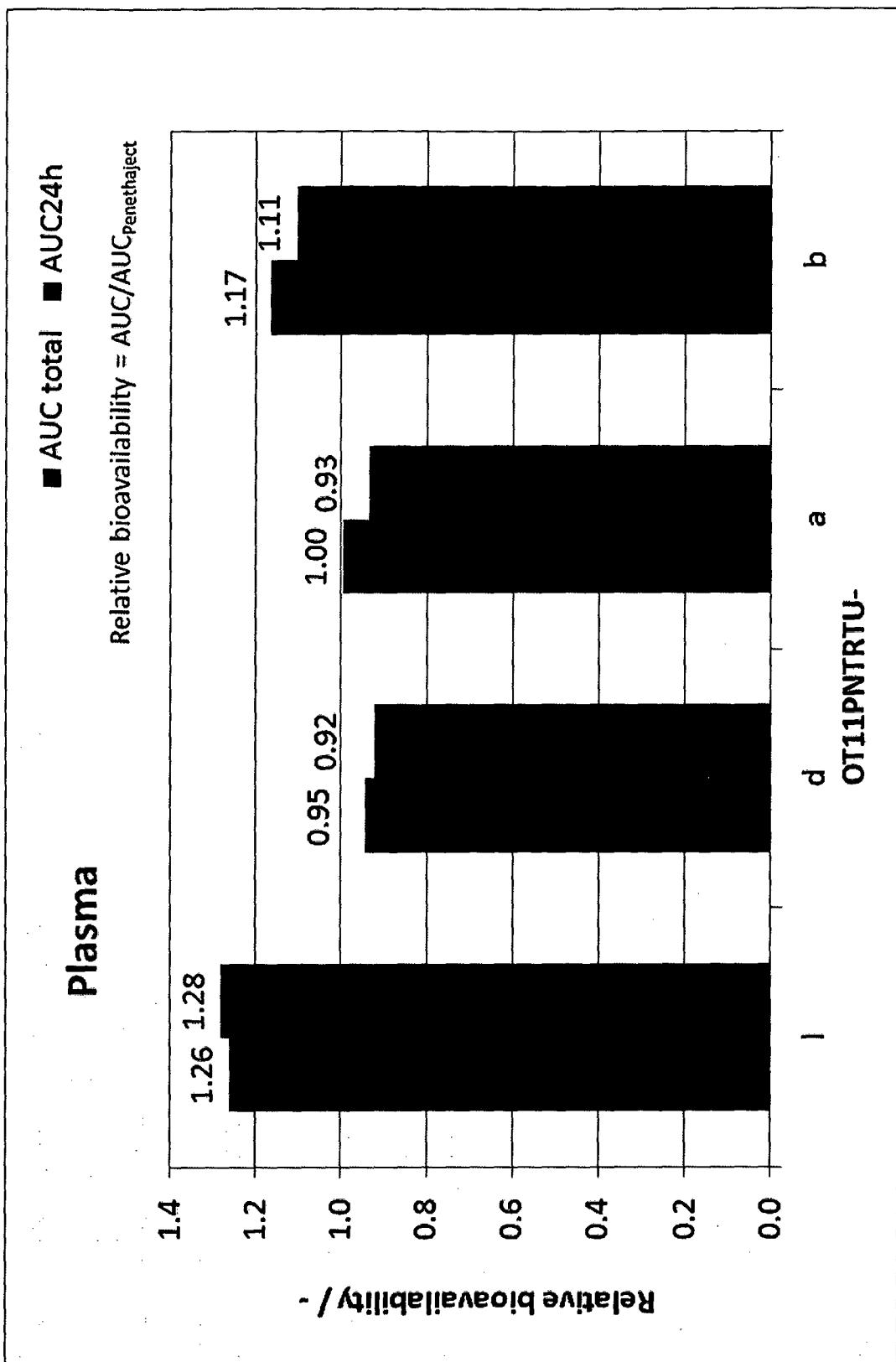


FIGURE 14

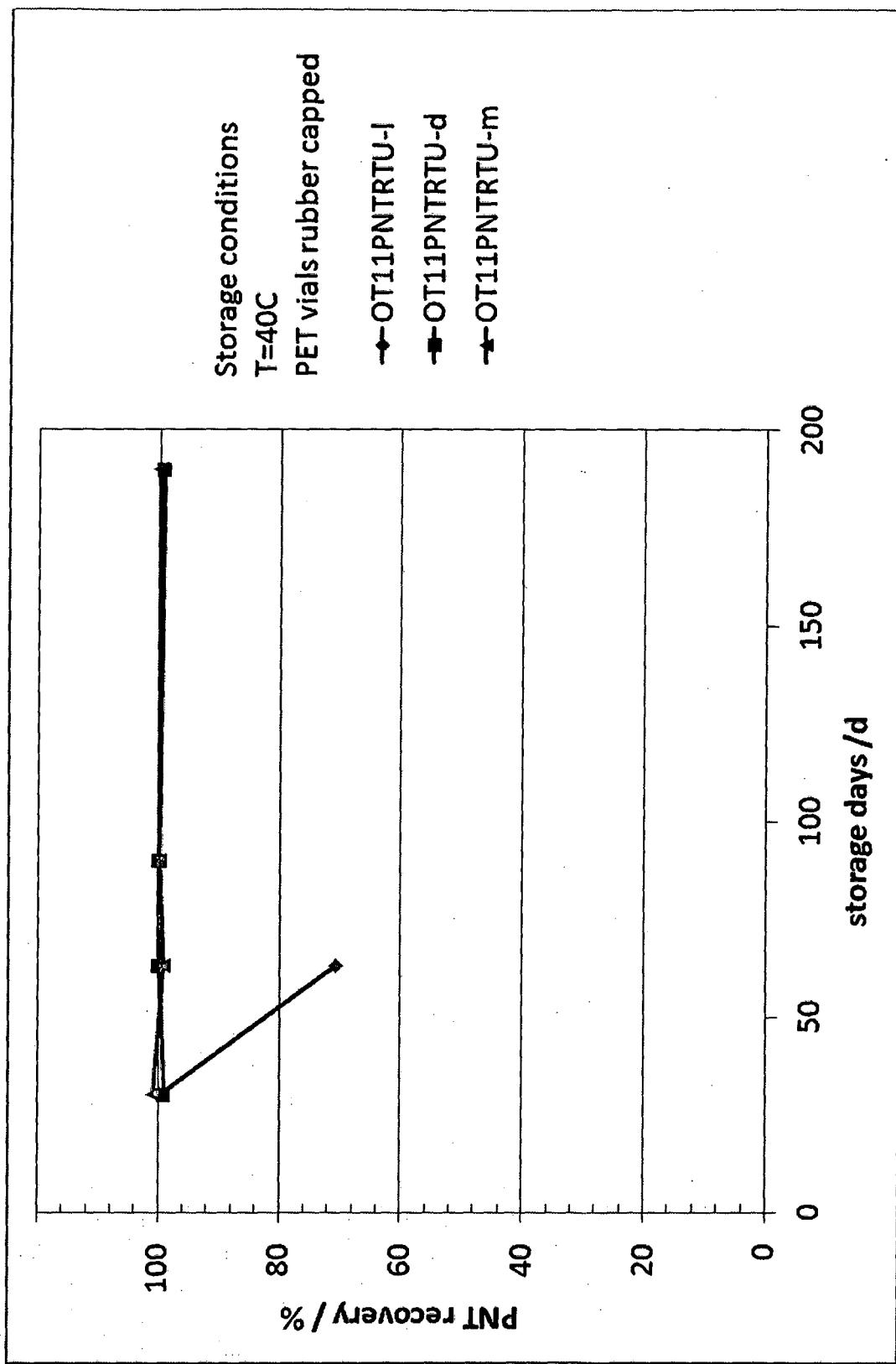


FIGURE 15

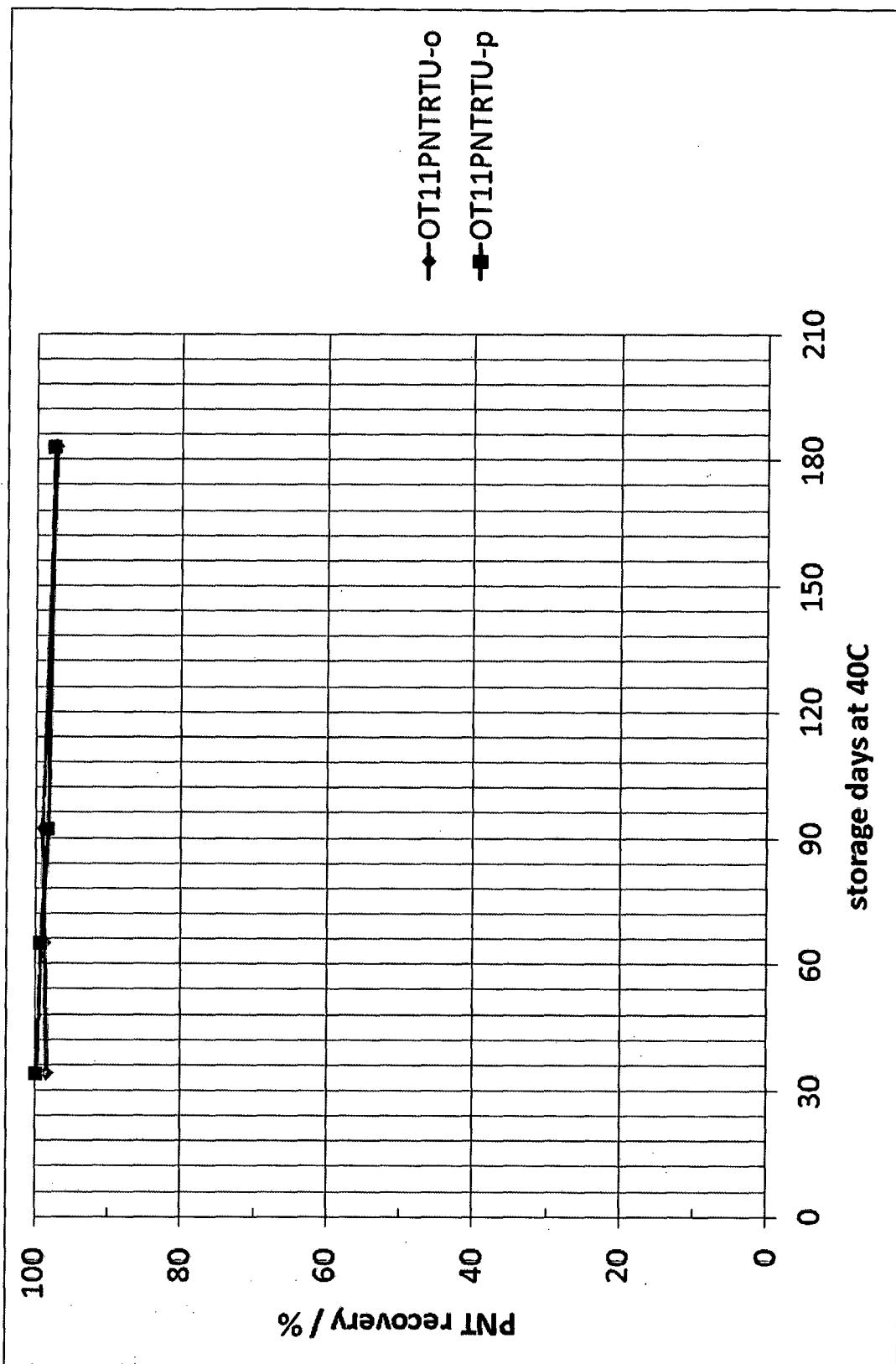
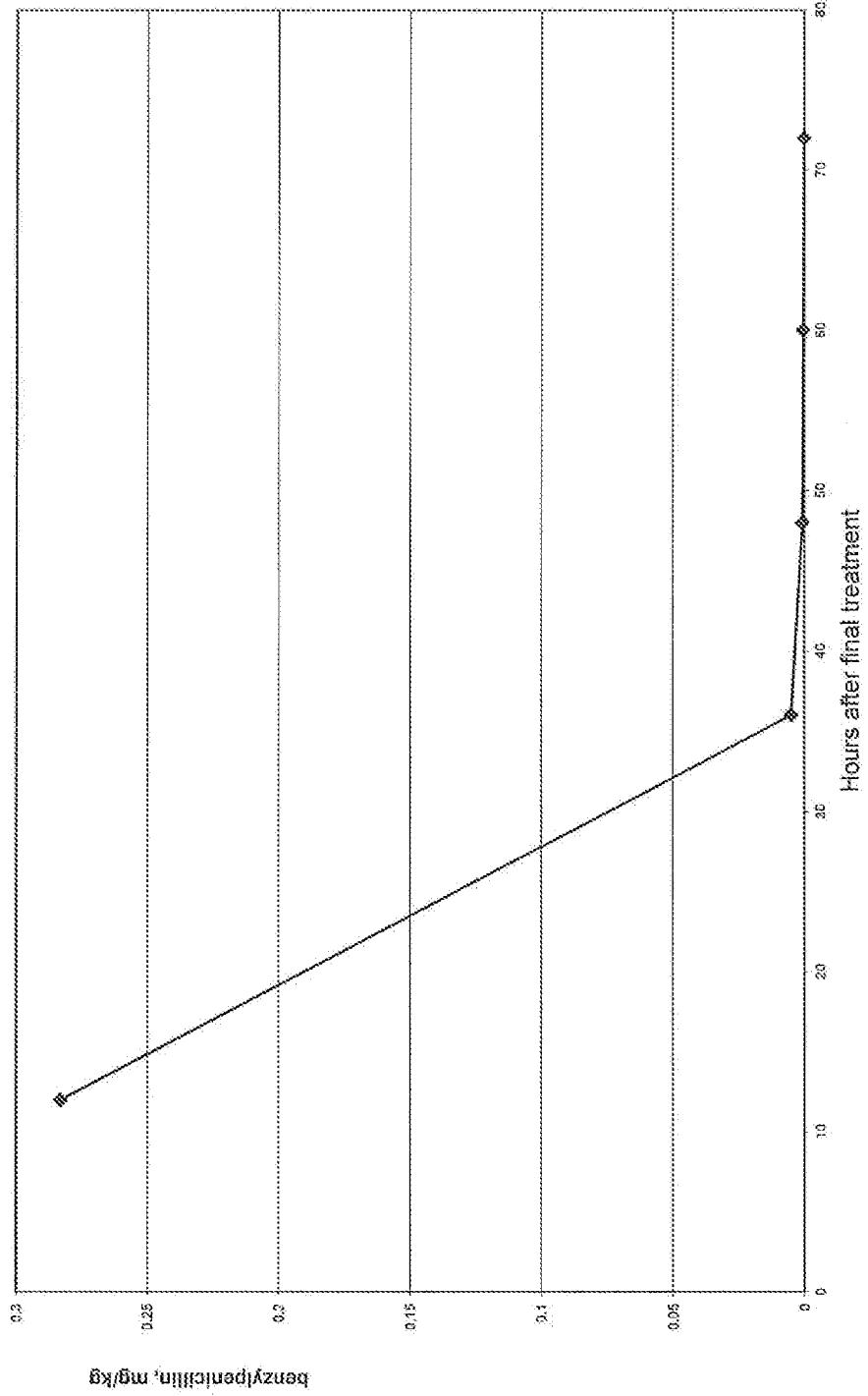


FIGURE 16

Depletion of benzylpenicillin in milk



Depletion of benzylpenicillin in milk

