A STANDARD PROTOCOL for DERIVATION and ASSESSMENT of STABILITY

Part 4 – Parenteral Nutrition

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A Standard Protocol for Derivation and Assessment of Stability

Part 4 – Parenteral Nutrition

1. Scope

This document is the fourth in a series of standard protocols to support NHS compounding units and purchasers to derive and assess stability data for pharmaceutical products made as Specials and those that are extemporaneously compounded. This protocol includes parenteral nutrition (PN) presented as Three-in-One (lipid-containing) admixtures or Two-in-One admixtures (with or without a separate intravenous lipid emulsion) and with or without micronutrients (trace elements and vitamins).

This document summarises evidence available for determining the shelf life of compounded PN. It is acknowledged that there are some limitations in the recommendations of this document namely:

Relatively few peer reviewed published PN stability studies have been conducted in the UK. The applicability of international studies to the local situation remains unclear. Most available data are limited by small study size and are often not reflective of contemporary UK practice. Moreover, the majority of PN stability studies have been conducted by the PN product manufacturers, either internally or in contract research laboratories. As such the data is frequently confidential, unpublished and non-peer-reviewed.

Consequently, the recommendations contained in this document are often based on expert opinion, which is generally classified as 'moderate to low' (GRADE process) or grade C (ESPEN guidelines for PN).

However this should be understood in the context of the realities of PN research in which the usual elements of well-designed pharmaceutical randomised controlled trials, notably blinding and randomisation, are not always possible. As such, lower grades of evidence, based on expert opinion, often represent the best level of evidence available and this does not necessarily invalidate the recommendations.

This document aims to:

- support NHS and commercial aseptic units during their assessment of stability data ahead of compounding PN, including the use of database resources as part of such assessments.
- support preparation of specifications for PN admixtures and design of tenders for PN services.
- provide a reference source for procurement and logistics staff when consideration is given to the purchase of unlicensed PN for inpatient and homecare use.
- describe the factors influencing PN stability, impact on shelf life and to provide an overview of assay methods utilised in the assessment of such.

In addition, this document offers support with regard to strategies for demonstrating suitable validation of the use of automated compounders and gravity fill techniques, and the testing of PN prepared using these methods. These issues are of fundamental importance to ensure that a formula has been correctly compounded ahead of it being entered into stability trials and can also be referred to when considering quality control analysis of compounded PN.

Furthermore, this document highlights considerations with respect to PN storage time whilst maintaining the cold chain, and stability assurances during infusion at standard room temperature and higher room temperatures.

2. Introduction

PN is unique in that, although it is a source of nutrients designed to support physiological processes it is also a Prescription Only Medicine made from licensed and sometimes unlicensed medicinal products.

PN is a complex mixture of components, and can contain over fifty separate chemical entities. The complexity of such admixtures requires careful consideration of factors that may affect stability, which include: overall admixture composition, mixing order of individual ingredients; mixing process (automated or manual / gravity fill); type of final container; quantity of air in the final container and/or dissolved within the final admixtures; and the quantity of air found in individual ingredients, exposure to light; temperature during storage; duration of storage. The inclusion of micro-nutrients (trace elements and vitamins) is almost always an essential part of PN admixtures but these can generate potentially hazardous by-products that need to be understood and their clinical importance determined[1].

To have a viable PN service there must be assurance that products will be assigned with an operationally and clinically relevant shelf life. Currently insufficient analytical techniques are available to evaluate the full spectrum of chemical and physical changes which might occur after mixing individual components to formulate a PN admixture and during subsequent storage, transport and administration. Adequacy of analytical methods should be carefully assessed. Requirements for stability and quality control testing should be risk-based.

The complexity of PN means that it is not possible to assign shelf lives based on the criteria often applied to other aseptically prepared products, such as the time for a certain percentage loss of one main ingredient or the levels of a single degradation product that may accumulate over time.

There are three basic types of PN admixtures used in clinical practice: lipid emulsion, either alone or with vitamins (which may be co-infused with a two-in-one admixture through a Y site); two-in-one admixtures containing amino acids and glucose with or without electrolytes and micronutrients; and three-in-one admixtures containing amino acids, glucose and lipid with or without electrolytes and micronutrients (often also referred to as All-In-One PN mixtures).

PN regimens for adult patients can be very different to those for paediatric patients, which can, in turn be very different to those for neonates. For example, there may be different ratios of components or quantities of macronutrients or electrolytes. In neonates, two-in-one admixtures with or without a separate lipid phase tend to be used. - Although two-in-one admixtures, of protein and glucose with a separate lipid phase, form the majority of bags used for neonates in the UK the use of three-in-one admixtures for children is increasing, especially in Europe. There has been data in the literature on three-in-one admixtures for children for some time. Component manufacturers can provide stability references for paediatric three-in-one regimens. One recent published study looked at three-in-one admixture stability for neonatal PN[2].

Micronutrients (trace elements and vitamins) are added to PN admixtures either during compounding or when aseptically manipulating a standard formula for a named patient, and stability assessment needs to be relevant to the process undertaken.

Sometimes simple infusates such as glucose with electrolytes are referred to as PN. There can be specific issues regarding the stability of micronutrient preparations in glucose 5% bags due to the unbuffered acidity of the glucose solution and the absence of stabilising / complexing amino acid solutions. Hence these preparations also need to be carefully assessed.

Part of the procurement process for aseptically compounded PN requires the appropriate assessment of supporting stability data, which is discussed in this document and a supporting summary checklist provided in Appendix 1.

3. Risk Management considerations

Due to the complexity of the formulations and production processes professional judgement is always an important aspect of shelf life assessment. Interpretation of stability data and correlation with specific formulations, local practice and conditions, and the needs of the patient are of paramount importance in minimising risk.

As stated above, PN is a complex mixture and it is not currently possible to assess the stability of all of the ingredients independently. All individual components in a PN formulation are stable at the time of manufacture and sterilisation in their original container.
However, when they are mixed together complex reactions can occur and the stability of the overall admixture needs to be assessed.

Some vitamins and some amino acids are inherently unstable in the final admixture. For example, ascorbic acid is highly prone to oxidation, although in becoming oxidised it can protect other components from oxidation. Another example of an inherently unstable component is vitamin A, which is highly photo-unstable without suitable light protection of the bag. Light protection is also important to ensure the stability of amino acids and lipid emulsions[3]. Consideration also needs to be given to light protection whilst the PN is passing through the administration set.

3.1 Compounding processes

The order and method of mixing are critical criteria for PN stability. It should be noted that most stability studies are conducted on PN compounded by gravity filling and manual additions. PN made from the same concentrations of individual components using an automatic compounder that can mix components within the tubing poses an added challenge which should be understood and considered ahead of data extrapolation.

A bulk or a pooling stage in the production of PN admixtures may be useful, particularly for low volume formulations (neonates / lipid solutions) and can have the advantage of enabling a bulk sample for chemical and microbiological analysis. However, this process itself may impact on stability, particularly if the formulation contains potentially incompatible components. Before embarking on a process that requires pooling risk assessments and process validation must be carried out in line with the recommendations of the MHRA Q&As for Specials Licence Holders[4].

3.2 Air in the formulation

The amount of air dissolved within the formulation and retained in the final product container will have a direct impact on stability of the mixture. Automated compounders may introduce more air than gravity filling and in either case as much air must be removed from the final bag as possible during preparation. Nevertheless, a quantity of dissolved air will remain within the bag which will be a source of oxidation. The quantity of air remaining in solution is temperature dependent since the solubility of air increases as temperature decreases.

Ascorbic acid, an antioxidant, is preferentially oxidised in the presence of other oxygen-sensitive ingredients. Adding an excess of ascorbic acid can therefore help to stabilise the formulation. The oxygen load from one typical litre of glucose or normal saline leads to a loss of approximately 50mg of ascorbic acid[5]. It is therefore common practice to recommend supplementation with, for example, 50mg of ascorbic acid per litre (as an antioxidant) plus 50mg to cover the UK recommended daily amount (RDA) for the adult patient.

Note that UK FSA recommends 40mg/d for healthy adults, but ASPEN, ESPEN and new AuSPEN PN guidelines recommend 100-200mg/d for adults. Commercial multivitamin preparations contain variable quantities of ascorbic acid (e.g. Cernevit® (Baxter) 125mg per vial and Solivito® N (Fresenius Kabi) 100mg per vial).

3.3 Final container choice

PN containers should never be over-filled because there must always be sufficient potential to remix the bag ahead of use and in the event of creaming.

Ethylene vinyl alcohol (EVA) plastic is semi-permeable to oxygen (and other gases) and hence has been largely superseded by multilayer oxygen barrier PN infusate containers. Use of oxygen impermeable bags largely prevent ingress of air and hence will reduce the destabilising effect of oxygen on micronutrients, amino acids and lipid emulsions and are recommended for use with all PN admixtures. These bags are also able to minimise the risk of activating air detection alarms during infusion.

Risks associated with the use of syringes as a storage device for compounded products should be weighed against the risk of over-dose from the use of larger volume containers. See section 5.1.2 for more detail.

3.4 Impurities

Impurities, such as aluminium, could be present in any individual PN ingredients before compounding and can impact on the safety and stability of the PN formulation. There is evidence that plastic containers leach considerably less aluminium than glass containers, particularly for organic ingredients such as calcium gluconate[6,7,8]. Note that aluminium levels in PN are more likely to be of clinical concern[9] than to be an issue for the stability of the admixture, especially for neonates.

3.5 Storage temperature

PN is almost always stored in the refrigerator (unless it is infused shortly after compounding). There are several issues to consider with the product cold chain process. The time it takes to cool a bag to refrigeration temperatures can be significant, particularly for large volumes (this will impact on the turnaround time for suppliers). Cold chain transport for PN is critical, and the risk of contact between prepared bags and frozen cold packs must be eliminated as this could allow localised freezing of the admixture and subsequent physical degradation.

It is vitally important to follow Good Distribution Practice[10] and to maintain the product within 2-8^{o}C during transport. There are various choices in terms of methods of ensuring compliance with the cold chain, whichever method is used it must be validated. Temperature cycling is likely to contribute to instability of the lipid emulsion and should, therefore, be avoided.

3.6 Infusion period

There are two main considerations for determination of the infusion period for PN, the chemical and physical stability and the microbial growth potential.

PN is normally infused over 24 hours or less and there are a number of guidelines that effectively limit infusion to this duration[11,12]. However, in some cases infusion periods have been extended to 48 hours in order to reduce risks of line infections during bag changes.
There is only evidence to support this in neonatal practice where infusion is via a suitable endotoxin retaining 0.22micron filter, in general manufacturers do not support an administration period beyond 24 hours in their stability statements, although certain products are licensed for a 48 hour period at room temperature once activated[13,14].

The in-use period of the stability study must also include sufficient time to warm the bag up to room temperature following removal from the refrigerator; this can take some hours for large volumes of solution.

During the in-use period it can be assumed that, for many adult patients and homecare patients the bag is at room temperature (25°C). Neonatal units tend to be kept much warmer than this and hence for these the in-use storage could be up to 30°C, which should be accounted for in stability studies and assessments.

4. Causes of Instability

The causes of instability of PN admixtures are considered below.

4.1 Lipid emulsion instability

Lipid stability considerations include both chemical and physical stability.

Chemical stability of lipid emulsions is still poorly understood and the clinical impact of the by-products of lipid peroxidation, such as MDA (malondialdehyde) is still under investigation. Lipid peroxidation is known to be accelerated in the presence of micronutrients, light, oxygen and temperature[15,16].
The routine use of oxygen barrier bags mitigates this process as does the light protection. The impact of light exposure during infusion is currently an active research area in neonatology[17].

Physical lipid emulsion destabilisation is a time-dependent phenomenon, although under certain conditions it can be rapid. The process begins with aggregation of lipid droplets and this is followed by creaming (potentially reversible) and flocculation leading to subsequently cracking of the emulsion (not reversible).

Modern lipid solutions generally contain a mixture of oils that are mixed before the emulsifier is added and therefore it can be assumed that individual droplets contain a mixture of oils[18].

When considering droplet sizing analysis for lipid emulsions small droplets may be an important consideration due to their large number in addition to larger, aggregated, droplets. This is because the smaller droplets may come together and aggregate more readily to cream and to crack. Therefore, any changes in droplet size distribution over time need to be carefully considered. Nevertheless, limits for lipid emulsion droplet size are normally established based on the development of enlarged lipid particles. Examples of the criteria that may be used are summarised in Table 1 below[19].

There is no consensus in practice on precise droplet size/count limits for all-in-one PN admixtures. There is a monograph in the United States Pharmacopoeia (USP) which does assign limits for lipid emulsions (see section 7.5), the use of which is being strongly advocated in some quarters for nutrient admixtures[20].

However, the monograph cited is intended for lipid emulsion analysis where products are licensed in the US and its application to PN admixtures is still controversial.

Table 1: Visual Classification of lipid containing Parenteral Nutrition (Cosslett[19])

Appearance	Definition	Acceptability
Homogenous	Uniform appearance throughout	+
Slight creaming	Cream layer <5mm, easily redispersed	+
Heavy creaming	Cream layer > 5mm with occasional aqueous layer at base, redispersion possible	+ *
Flocculated	Not redispersible	-
Cracked	Free oil present	-

* This is acceptable only as an extreme of any matrix.

4.2 Precipitations and micro-precipitations

Formation of insoluble phosphates of calcium and/or magnesium is a well-known risk with PN and therefore their concentrations in the final formulation are generally limited and different salts are used to further minimise precipitation risk. For example, the use of an organic phosphate source such as sodium glycerophosphate has become widespread, however some commercial amino acid sources contain inorganic phosphate. The incompatibility between calcium and phosphate or sulfate/or magnesium and phosphate is a very real issue during the compounding process especially if an appropriate mixing order of components is not used, or if the use of automated compounders results in their mixing within the plastic tubing sets used during PN preparation. With three-in-one formulations it can be difficult or impossible to detect precipitations hence with these admixtures the risks are greater.

The final pH of the admixture and the pH of the amino acid solution used can significantly impact on the likelihood of calcium phosphate precipitation, hence the maximum safe concentrations are specific for each amino acid source. Lipid bound phospholipid does not impact on calcium phosphate stability

The solubility of calcium phosphate and calcium sulfate decreases with increasing temperature, increasing the risk of precipitation. This can be of particular concern in neonatal units where bags may be infused at 30^{o}C.

There are other elemental components of PN which may cause micro-precipitates seen as a haziness within the solution, these are difficult to spot using conventional means and are generally noticed using the Tyndall Beam Effect[21].

Iron phosphate can form a micro-precipitate due to the nature of the phosphate in Synthamin®. Hence if iron is to be added to a Synthamin® containing admixture electrolyte free (Synthamin® EF) should be used and the phosphate (as organic) and other electrolytes added as required. In some cases, the addition of ascorbic acid to the formulation also appears to prevent the problem occurring[22].

A complex microprecipitate, can also be formed by interaction between copper and a degradation product from sulfur containing amino acid solutions (cysteine) believed by some to be copper sulphide and others to be copper cysteinate[23].

Selenium plus high concentrations of ascorbic acid is another theoretical risk of micro-precipitation that has been reported in the literature[24,25]. However, other studies have suggested that it is not expected to be seen in routine practice using standard doses of the two components and where the pH of the final solution is above pH 5[26,27,28].

4.3 Vitamin instability

There are two main causes of vitamin instability in PN: oxidation and photodegradation.

Oxidation results from the presence of oxygen, which is introduced into the bag during preparation. This includes air which is dissolved in certain PN ingredients (e.g. glucose infusion, electrolyte additives) or the air able to diffuse through the PN bag into the infusate during storage. Such reactions are time-dependent and therefore the rate of reaction and extent of losses will vary according to the composition of an individual PN formulation.

Ascorbic acid is perhaps the PN component that is the most reactive with oxygen and so if present it will be preferentially oxidised rather than other components. This reacts with the available oxygen in the infusate, which may protect other components within the formulation. Following the addition of ascorbic acid there is a rapid reaction with any oxygen present within the bag, which is generally complete within 24 - 48 hours assuming further ingress of oxygen into the infusate is limited by the use of an oxygen barrier bag. In this case, the total loss of ascorbic acid will depend almost entirely on the amount of oxygen present within the PN infusate immediately after compounding. If an oxygen barrier bag is not used the ascorbic acid will continue to be oxidised beyond the first 24 – 48 hours[29].

Ascorbic acid does eventually degrade to Oxalic acid, which could in theory react with calcium to form insoluble calcium oxalate but this is only of consideration in extreme circumstances where significant ascorbic acid degradation is expected. This would not be the case if oxygen availability is minimised using oxygen barrier bags and removing excess air from the bags[30,31,32].

Photodegradation of PN components such as vitamins A and E occurs when PN admixtures are exposed to daylight (UV / short wavelength visible light). Vitamin A is the most important light sensitive vitamin, the rate of degradation is affected by the presence of lipid emulsion (which may block some of the light), the depth of the solution, the time of day and the local weather. Vitamin E can also be affected by light due to a photo-oxidation reaction but it is generally protected from this by the presence of excess ascorbic acid[33]. Light protection of PN is paramount to maintain stability of these ingredients.

4.4 Other component chemical instability considerations

Specific amino acids may also react with dissolved oxygen, especially cysteine (which may lead to the generation of hydrogen sulphide) and tryptophan, whose degradation products can cause darkening of the PN mixture.

The Maillard reaction where the reactive carbonyl group of a sugar reacts with the nucleophilic amino group of amino acids to produce a variety of substances normally resulting in browning of the solution has been reported as an issue[34] but is not likely to be significant at the normal temperatures of compounding storage and administration of PN.

4.5 Additions of drugs into PN and Y-site compatibility issues between PN and other infusates

The addition of drugs into PN or mixing of PN with other infusates via an administration set / Y-site is discouraged since it can often cause compatibility issues. There is some evidence to support ranitidine and cimetidine addition to certain formulations[35,36] but suitable data and specialist knowledge should be available before deciding to add any drugs to, or mix any drugs with, PN.

4.6 Incompatibilities with line locks

Line locks may be incompatible with PN (e.g. heparin with lipid-containing PN) and require careful flushing protocols.

5. Stability Assessment

5.1. Containers

The containers used in stability studies should match those that will be used for the product, all types of container to be used should be tested.

5.1.1 Parenteral Nutrition Bags

Generally oxygen barrier bags should be used for all bespoke PN unless a very short shelf life is assigned. Multi-chamber bags once removed from their overwrap may not offer a full oxygen barrier and under normal circumstances once mixed and vitamins added the shelf life should be restricted according to available data.Once the multi-chamber bags have been removed from their overwrap then the in-use shelf life should be assigned whether or not the vitamins and trace elements are added straight away.

In some cases expiry dates can be extended by addition of excess ascorbic acid, repackaging into an oxygen impermeable outer bag and removing the air from this together with the inclusion of an oxygen scavenger within the overwrap.

It is important whatever containers are used that as much air as possible is removed from the bag before final seals are added.

5.1.2 Syringes

In some units syringes are used for the containment of the lipid emulsion in neonatal PN. Syringes on the market do not have registration as storage devices, hence when used as storage containers they must be fully validated including for microbiological integrity and physical robustness. Please refer to the 'Protocol for the integrity testing of syringes'[37] for further information.

The syringe and closure system should be fully defined and the data generated will be specific to this system. It is desirable to use luer lock syringes, two piece polypropylene syringes may also be suitable and may have lower extractive levels, however, past history indicates that there may be more issues with the integrity testing[38].

An assessment of the level of extractives from syringes containing lipid phase should be carried out and the implications understood. The British Pharmacopoeia (BP) monograph for plastic syringes[39] provides useful guidance and limits for syringe extractives, however, it should also be considered that lipid emulsions may leach different materials from the syringe when compared to aqueous solutions.

It is preferable that syringes, with the plunger attached, should not be filled to higher than 85% of their marked capacity, to prevent undue plunger movement and thereby compromise microbial integrity during shipping and distribution. This should be reflected in the design of the stability trial.

Note that the lipid phase of PN has shown itself to be a very good growth medium for micro-organisms and hence the integrity of the container is of paramount importance.

5.2. Storage Conditions

PN once compounded is almost exclusively stored in a refrigerator, cooling times and time to naturally warm up to infusion temperature together with the infusion time (normally at room temperature 25^{o}C) should all be considered when designing a stability trial. Remember that small volumes will cool and warm more quickly than larger volumes; hence ideally the full volume required should be studied. It is standard practice to allow 2 hours for PN to warm sufficiently following cold storage before administration is begun.

Generally only two storage conditions are required both being protected from light.

a) Refrigerated in the absence of light (5^{o}C± 3^{o}C) storage
b) Room temperature (25^{o}C ± 2^{o}C) infusion time plus cooling / warming / excursions

However, neonatal PN may experience higher temperatures during the infusion period with some units being at 30^{o}C, which needs to be considered when designing a stability study for neonatal formulations. Furthermore, homecare PN patients may have an ambulatory pump and the PN may therefore be closer to the patient (e.g. in a backpack) and so a higher in-use temperature may need to be assessed.

For PN the room temperature / in-use scenario data can be obtained by storing for a period at room temperature / higher temperature at the end of the study, this would normally be limited to 24 hours or 48 hours[11,40,41,42,43,44]. This will reduce the costs of a study compared to an independent room temperature / higher temperature study being carried out and give more clinically relevant data.

PN should be routinely protected from light during infusion (see above). If the product is likely to be exposed to light during infusion then this is likely to be a complex scenario and one would also need to assess impact of the type and level of light and consider exposure in the administration set.

PN is always an aqueous solution or emulsion and hence apart from an assessment of the moisture loss from the container there is no need for humidity control in the stability study. Humidity variation will not impact on product stability.

5.3 Orientation of containers during stability studies

Solutions should be stored horizontally for the duration of refrigerated storage. During the simulated in-use period the containers should be stored vertically, this will enable better visual assessment of any creaming or cracking of the emulsion. It is important in practice that PN is stored in this way.

5.4. Matrices

Stability of PN is generally determined by a matrix approach that considers the composition of an infusate. A range over which the individual formulations are stable is determined and this informs the limits of the matrix. Assuming stability can be demonstrated at the edges of the

matrix area then data may be interpolated to give a degree of stability assurance within the matrix.

If a formulation falls outside of the matrix then a specific stability study or extrapolation of further studies to extend the matrix will be necessary. In the interim period a formulation meeting known data should be used or if absolutely necessary then a risk-based decision needs to be taken by the prescriber and accountable pharmacist.

5.5 Storage Period / Study Length

Due to the complexity of PN, and the risk based approach to assignment of shelf life for most formulations the minimum shelf life required for the product to be viable should be allocated. For stock bags of standard formulations this may be up to 90 days, but for individually tailored bags, where stability data is based on interpolation from a matrix, shelf lives should not normally exceed 16 days for hospital inpatients and 30 days for homecare patients, even if additional data exists. This allows provision of a viable service to patients allowing for compounding and transport and also to cover exceptions such as patient holidays.

Stability studies should reflect these periods and sampling normally carried out at the beginning and end of the refrigeration storage period, and then after the simulated infusion time with an added margin of error e.g. a further period at room temperature to allow for variations in clinical practice.

5.6. Sample Numbers

In line with other stability trials then stability samples should be prepared and assessed in triplicate, ideally using separate batches of critical starting materials. However, the decision on sample numbers may be dependent on the amount of information available on similar admixtures. It is acknowledged that there is much historic data which has been generated using a single sample and it would not be practical to invalidate that data but new studies should consider a higher number of samples where appropriate.

5.7 Preparation / production process

The production process used for PN can impact the stability of the final formulation, and problems such as precipitation can arise during the mixing process. Automated compounders need to be carefully validated in this context since a majority of the PN mixtures used for stability studies have been compounded by gravity fill.

An impact assessment should determine whether stability data could be extrapolated to mixtures compounded with automated compounders, the mixing order and the amount of mixing of solutions in the tubing are likely to be critical parameters.

Air must be removed from compounded bags to leave as little as possible to prevent oxidation of the components.

5.8 Quality Control Assessment of stability samples

It is essential to ensure that stability trials are undertaken with known formulations, there are various options for the quality control testing of PN admixtures which should be considered as a compounding accuracy check ahead of the start of the stability trial. The extent of any such QC testing should be based on a risk assessment and other in-process controls will form part of this assessment. The tests referred to below can also be used for routine quality control testing of compounded PN or as part of a validation protocol for the assessment of accuracy of automated compounding equipment, carried out on commissioning and then routinely to ensure continued

compliance. Note that these are not stability indicating methods and are not part of the stability assessment itself, only a confirmation of formulation accuracy.

a) Electrolyte levels

Sodium and potassium levels can be measured using Atomic Emission (AE) spectroscopy or Flame Photometry (FP). Levels of these and other key cations including calcium and magnesium can be measured using Atomic Absorption (AA) spectroscopy or Inductively Coupled Plasma (ICP) spectrometry. ICP can also be used to measure many of the other elemental constituents of PN including certain non-metals, trace elements and also impurities such as aluminium.

b) Refractive Index

Refractive index can be used to confirm the general composition of a PN formulation and will be mainly influenced by the glucose and amino acid concentrations in the formulation. High levels of electrolytes and organic salts such as gluconate and glycerophosphate will also have an influence on the final refractive index. A matrix calculation can be used to generate a theoretical refractive index and to compare with the measured level.

For lipid-containing admixtures the lipid will need to be filtered out (using a 0.2micron or similar filter) or centrifuged ahead of testing.

Osmolarity or specific gravity can also offer some indication of the correct compounding of the admixture. Note that all of these methods are non-specific and the measured values are influenced by all components present in the mixture, hence they are only indicative.

c) Chemical tests

The BP test for phosphates is suitable for determination of the phosphate salt used in compounding[45]. Lipid bound phospholipid gives a negative result within the normal test timeframe (no colour change). If required the total amount of phosphate can be assessed by chemical methods.

More detailed chemical testing can be carried out as required using techniques such as High Performance Liquid Chromatography (HPLC) and testing for specific amino acids and vitamins. This would not be deemed necessary as an assessment of compounding accuracy as the above tests together give a high degree of assurance.

6. Stability Testing Protocols

The minimum testing protocol for stability assessment of PN should include

a) Colour, clarity, creaming and cracking and particulates, analysis of PFAT5 (percentage of fat residing in globules larger than 5µm) and/or lipid droplet size (see section 7.5 below)

b) pH

c) Assessment of sub-visible particle levels for two-in-one admixtures, for example light obscuration particle counting, turbidometric techniques, Microflow Imaging or other particle size analysis. Some imaging techniques can determine differences between lipid and other particles and so will be of some use in three-in-one mixtures too.

d) Assessment of degradation of known unstable ingredients such as Ascorbic Acid and the protection that Ascorbic Acid can have on the rest of the formulation.

Additional parameters which may need to be considered include

a) Moisture loss (this can be carried out by weight check after storing in a refrigerator or in order to better compare container characteristics at ICH (International Conference for Harmonisation) low humidity storage conditions). This is unlikely to be significant for the periods of storage for compounded PN.

b) Container leachables and extractables - likely to be a property of the container and independent of the formulation, and hence will not form part of routine PN stability studies.

7. Test Methodology

There needs to be good understanding of the inter-relationship between the tests selected and the interpretation of the data generated. A range of tests will be required to fully assess the stability of a specific formulation or a matrix, details are given below. There are currently no Pharmacopoeial standards and hence techniques used are likely to vary between laboratories.

7.1 Visual Characteristics

Appearance of solution / emulsion, colour, clarity, levels of creaming and the ability to be remixed, emulsion cracking and absence of visible particulates. Visual examination is a vital part of stability assessment for PN solutions.

No significant colour change is expected within the storage period of a PN solution, if protected from light. However, in particular for vitamin-containing admixtures, colour changes can occur. Colour can be generated by highly coloured components (ingredients or degradation products) in very small amounts and is therefore only loosely correlated with stability. Colour change can be determined by comparison with standards such as Lovibond Reference Standards.

For visible particulates / precipitations in 2-in-1 admixture then standard viewing equipment such as outlined in the BP[46] and polarised light viewers are suitable. Fibre optic lighting (Tyndall Beam Effect) can be useful in the identification of micro-precipitates. Multivitamin products can cause a haze in the solution on adding, which may clear over time, this is not a sign of an instability issue but may interfere with initial testing results.

Table 1 indicates acceptance criteria for lipid emulsion stability. Cream layers are not always easy to see and tend to be white until significant physical deterioration takes place causing the yellowing often seen in illustrations.

7.2 Re-dispersibility

If there is some evidence of creaming in a bag the potential for re-dispersion needs to be assessed. This would normally be based on inverting the bag end over end and ideally the emulsion should re-disperse in ten rotations, with an outside limit of twenty rotations[19], laboratories may have other equivalent standards.

7.3 pH

Whilst pH is critical to the stability of a PN infusate, significant changes in pH are rare since the amino acid solutions used are good buffers. Nevertheless, this means that pH changes during a study can indicate a critical stability failure. In general, a limit of +/- 0.5 pH units should be applied to ensure there has been no significant change in the product.

7.4 Particulates

For Lipid-free PN a sub-visible particle count using the BP Light Obscuration methodology[47] is one option for assessment of particle load and the formation of precipitates (limit as per BP). Alternative options include turbidometric determination (limits usually no change greater than +/- 0.5 NTUs). In order to better understand the reactions occurring it is recommended that any precipitate present is isolated and identified using suitable methodology.

7.5 Lipid emulsion droplet size

Zeta potential was previously used in the determination of likelihood of droplet coalescence but there is little evidence for its usefulness. Stability assessments are therefore instead based on visual examination and particle sizing systems.

Light microscopy using normal light or fibre optic lighting can be a useful technique, which generally involves the use of clean blood counting slides (free of oils from the analyst's skin). Care must be taken since cover-slips may squash lipid globules and change their size and shape. Laser Diffraction is another useful technique, which uses an instrument optimised for intravenous emulsions.

The limits for microscopy techniques are based around ensuring no significant change over the storage period with limits for various particle size ranges and consideration of the shape of the droplets.

There are some specific limits set out in the USP monograph <729> [48] regarding analysis of particle size in lipid emulsion, the monograph is intended to apply to lipid emulsions rather than full PN admixtures. Although the principles of PFAT5 and limiting numbers of globules greater than 5µm in PN admixtures is sound there are concerns over the use of light obscuration techniques in order to measure this. Furthermore light obscuration techniques will not pick up the smaller lipid droplets which may have an important role in indicating infusate instability.

The USP standards are set out below but have, to date, not been officially accepted in the UK or Europe, instead a combination of assessments methods based on visual, microscopic and a particle size analysis (e.g. laser diffraction, light obscuration) have been used routinely to determine lipid droplet stability in PN.

Chapter <729> of the United States Pharmacopeia (USP)[48] has two methods for the determination of globule size distribution in soybean lipid based injectable emulsions, Method 1 is based on light scattering methods with a choice of using classic light scattering or Dynamic Light Scattering. Use of either option results in a limit for the intensity weighted mean droplet diameter (MDD) of less than 500nm irrespective of the concentration of the dispersed lipid phase.

The second USP method is measurement of the large globule content by a light obscuration or extinction method. The volume-weighted, large diameter fat globule limits of the dispersed phase, expressed as a percentage of fat residing in globules larger than 5µm (PFAT5) for a given emulsion must not exceed 0.05%. The adequacy of this method for complex mixtures such as PN admixtures has been questioned and results should be taken with care and alongside other assessment criteria[49].

7.6 Chemical stability

The stability of parenteral admixtures is documented using various physico-chemical methods. Changes during storage of admixtures have been identified to be mainly physical in nature, such as precipitation, physical destabilisation of the emulsion etc. Normally, chemical stability is not specifically studied due to that nutrient components remain stable during short-term cold

storage, which is the condition for most practical applications of parenteral admixtures. Furthermore, when chemical stability studies on parenteral admixtures have been performed it is generally found that the stability profile can be predicted based on the behaviour of single component nutrition products. Based on these considerations as well as extensive model studies, most companies do not routinely analyse for chemical stability during compatibility testing. However, during the course of long-term storage of an admixture, it is of course conceivable that chemical changes could occur.

a) Nutritional components

Traditional HPLC may have a role with some PN ingredients such as vitamins and amino acids, and therefore it is important to understand the degradation pathways for these components, particularly whether any harmful degradants (to the patient or to stability of the rest of the PN admixture) are formed. Methods are currently being worked up for combined analysis runs for vitamins and also for amino acids[19].

Potentially 5-HMF (Hydroxymethylfurfural) may form as a degradation product from Glucose solutions, this can be measured using HPLC but in reality is not known to be a problem in PN and routine assessment would not be expected. Furthermore it may be a technique of use in looking at peroxidation markers arising from the lipid emulsion.

b) Added drugs

HPLC is also likely to be the method of choice for analysis of drugs added to PN admixtures, but the practice of adding drugs is not recommended and needs to be fully assessed and understood before being considered. A Standard Protocol for Deriving and Assessment of Stability, Part 1 - Aseptic Preparations (small molecules)[50] should be referred to for further advice on stability of drugs added to Parenteral Nutrition.

8. Acceptance Criteria

The acceptance criteria are explained under individual tests but generally for PN these are based on no or limited change over the storage period, an exception maybe with components such as ascorbic acid where levels can be assayed, although even here significant levels of degradation are often acceptable.
Statistical analysis is generally not useful in assessing shelf life for PN.

9. Supplier matrices and computer generated stability statements

Many PN preparation units rely on matrices supplied by the ingredient Marketing Authorisation (MA) holder either as paper documents or as computer systems generating stability statements to inputted formulations. These are useful resources but appropriate validation should be sought or undertaken on the system, ideally using known stability problems to ensure that the output of the calculations is correct.

The use of a computerised system should supplement and not replace the professional judgement of a member of staff skilled in formulation and stability assessment. Clinical and technical services pharmacy staff need suitable training and understanding in the issues affecting PN stability in order to assess the information provided to them.

Note that generally these resources rely on using at least both main ingredients (lipid and amino acid) from the same supplier, and often vitamins and trace elements. Mixing components from more than one Marketing Authorisation holder may be outside the scope of the available stability

matrices and is generally discouraged by the manufacturers. It should, however, be acceptable if there is suitable stability data available.

10. Change Control

Due to the nature of PN then any changes to formulation and to production method must be fully assessed as part of a robust change control procedure. The introduction of new automated filling techniques is a particular risk which will require total understanding of mixing orders and potentially a re-evaluation of shelf lives of the PN formulae compounded using the automated equipment.

Formal change control is not required before minor changes to formulations within a stability matrix are made.

11. In Use stability evaluation

PN admixtures bags should be examined when they are issued to patients and nursing staff and homecare patients should be encouraged to carry out a macroscopic examination of the bag before use. Any problems identified during this examination should be fed back to the supplier of the stability data or matrix and the shelf life assigned to the admixture should be reviewed.

In use blocking of the filter should also be considered a potential indicator of compromised stability and a root cause analysis undertaken.

Glossary

ASPEN – American Society for Parenteral and Enteral Nutrition

AuSPEN - Australasian Society for Parenteral and Enteral Nutrition

ESPEN - The European Society for Clinical Nutrition and Metabolism

GRADE process – A systematic and explicit approach to making judgements about quality of evidence and strength of recommendations.It was developed by the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) Working Group,

Lovibond Reference Standards – a series of colour standards for visual comparison of the colour of solutions

NTUs – Nephelometric Turbidity Units

PFAT5 - percentage of fat residing in globules larger than 5µm

Two-in-One mixtures, which contain amino acids, glucose, electrolytes but no fat emulsion

Three-in-one mixtures which contain all of the above plus fat emulsion (often referred to as All-In-One PN mixtures).

Tyndall Beam Effect - the visible path of light produced by the scattering action (Tyndall effect) of the particles in a colloidal solution on a beam of light passed through it.

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Document History	Issue date and reason for change
Version 1	Issued May 2016
Version 2	
Version 3	
Version 4	

Appendix 1

Checklist for assessment of stability data for procured Parenteral Nutrition (Specials)

The following checklist is provided as a guide to assessing the suitability of procured aseptically compounded (from sterile injections - Specials and licensed starting materials) Parenteral Nutrition products from the stability assessment viewpoint. This should be used alongside other assessment tools for unlicensed products.

Preparation:……

Supplier / Manufacturer:…….

1) Formulation	1.1) Is the formulation specified in the product specification including any concentration restrictions?	Yes (go to 1.2) / No (return to supplier for specification)
	1.2) Is the formulation fit for purpose and for the patient / patient group?	Yes (go to 1.3) / No (source a suitable formulation)
	1.3) Is the preparation made by gravity fill, additions to a standard bag or using an automated compounder?	Yes / No (Record and proceed)
2) Shelf life assigned	2.1) What shelf life is assigned by the compounder?	
	Is the shelf life based on	
	2.2) the recommendations of the licensed PN components manufacturer?	Yes (go to 3) / No (go to 2.3)
	2.3) a specific stability study (in-house or supplied by PN component manufacturer)?	Yes (go to 3) / No (go to 2.4)
	2.4) an expert assessment of stability based on a published study?	Yes (Go to 3)/ No (go to 2.5)
	2.5) Is it based on extrapolation of data from either a published study or a statement provided by the PN component manufacturer?	Yes – ask the supplier for their risk based assessment for this process.
3) Interpretation	3.1) Is the data supplied applicable to the formulation and method of preparation?	Yes go to 3.2 / No ask the supplier for their risk based assessment for extrapolation to their processes
	3.2) Is the shelf life suitable for purpose i.e. practical for the required use but not excessive? Recommendations Maximum 90 days for compounded standard formulae without vitamins / minerals Maximum 30 days for compounded standard formulae with vitamins / minerals (but see text) Maximum 30 days for patient specific formulae for homecare patients Maximum 16 days for patient specific formulae for hospital inpatients	If compliant then specialist review of data and information supplied. If not then the data and shelf life should be subjected to expert review

Summary of risks

Assessment of stability study for

……..

The data supplied: Provides assurance that the product will be suitable, safe and efficacious / does not provide suitable assurance

Approved:…….Date:……

Additional risk reduction measures