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HPLC method for levothyroxine quantification in long-acting drug delivery systems. Validation and evaluation of bovine serum albumin as levothyroxine stabilizer

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ABSTRACT

Deficiency of thyroid hormones (hypothyroidism) is treated with oral levothyroxine (LEVO). However, the effectiveness of oral administration is highly dependent on the co-administration of food and other drugs. This factor, in combination with the chronic nature of this condition, mean that there are concerns with patient compliance. Development of long acting formulations to treat hypothyroidism could potentially solve this problem. However, LEVO instability in solution could be problematic. In order to develop long acting LEVO delivery systems in vitro drug release experiments should be carried out. However, short term LEVO stability in aqueous solution will prevent this. BSA was used as a stabiliser for LEVO; extending the stability of the drug in aqueous solutions from a few hours to 2 weeks. In order to achieve this, the required concentration of the protein was 0.1% w/v. Subsequently, an HPLC method capable of separating LEVO from the protein was developed and validated following ICH guidelines. The analysis was carried out using a reverse phase HPLC method on an Agilent 1220 Infinity II LC system. The column used to achieve separation was a Zorbax Eclipse plus C18 (95 Å pore size, 250 mm length x 4.6 mm internal diameter; 5 μm particle size). The mobile phase used was composed of acetonitrile and 0.1% trifluoroacetic acid at a ratio of 50:50% v/v. UV detection of LEVO sodium was carried out at 225 nm. The retention time for the drug was 6.6 minutes. The method showed a limit of detection of 0.03 μg/mL and a limit of quantification of 0.09 μg/mL. Finally, this method was used to evaluate the release from implants containing 20% w/w of LEVO. These devices were prepared using a solvent casting method with poly(caprolactone) and LEVO. These devices showed an initial burst release over the first 3 days. Subsequently, they were capable of providing a linear release rate over the following 25 days.

1. Introduction

Hypothyroidism is a medical condition characterised by underactive thyroid gland. Consequently, patients suffering from this condition present low levels of thyroid hormones. This condition is the most common thyroid ailment affecting over 1 million people in the UK and up to 5.3% of the population of other European nations [1,2]. Thyroid hormones play a key role on the control of several physiological processes including brain development, oxygen consumption and heat production [3]. Accordingly, deficiency of thyroid hormones leads to symptoms such as weight gain, cold intolerance or chronic fatigue [4]. It is important to note that long-term untreated hypothyroidism could lead to myxoedema coma and cardiovascular complications that could result in premature death [5,6].

Hypothyroidism is treated using oral levothyroxine (LEVO; also known as T_4) sodium [7]. Oral LEVO administration presents challenges whereby food [8] or other medications [9] could lead to LEVO absorption problems. Moreover, as hypothyroidism is a chronic condition, its management relies heavily on patient compliance. A potential alternative to improve the management of hypothyroidism is the use of long-acting drug delivery systems. These systems can be injected/implanted within the patient body and provide sustained drug release [10,11].

LEVO long-acting drug delivery systems have been described before [12]. However, testing this type of system is challenging due

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to LEVO instability. Instability of LEVO sodium is well documented and it has been reported to be susceptible to environmental factors such as light, air, temperature, pH, and humidity, among others [13]. As a result of these stability issues, there are significant manufacturing challenges associated with designing, producing, and testing a LEVO sodium containing product which must be considered. Degradation in the solid state occurs mostly by deamination, whereas, degradation in solution occurs mostly by deiodination [14]. It was important to consider these stability considerations in both the manufacture of the implant but also in the design of *in vitro* release studies. In order to stabilise LEVO in solution for long-term in vitro release, organic solvents are required. This work describes the use of a simple release medium containing a protein, bovine serum albumin (BSA), as a stabiliser and a conventional RP-HPLC method to address LEVO stability issues for medium to long-term release studies. This method was tested in the release of LEVO from polymeric implants over a period of 4 weeks.

2. Material and methods

2.1. Materials

LEVO sodium pentahydrate (purity: ≥99%) was obtained from Enke Pharma-tech (Cangzhou, China). On the other hand, trifluoroacetic acid (TFA) (purity: ≥99%), acetonitrile (HPLC grade) and bovine serum albumin (BSA) lyophilised powder (purity: ≥ 96%) were obtained from Sigma-Aldrich (Dorset, UK). Dichloromethane (DCM) was obtained from Merck (Darmstadt, Germany). Poly(caprolactone) (PCL) (CAPA 6506)(MW = 50,000 Da) was kindly donated by Perstorp (Malmö, Sweden). Finally, Silastic® S, a silicone rubber and curing agent mix (mixture ratio 10/1) was obtained from Thompson Bros. Ltd. (Newcastle Upon Tyne, UK).

2.2. Stock solution and working standards of LEVO sodium in 100% ethanol

A stock solution of LEVO sodium was prepared by dissolving the appropriate mass of LEVO sodium in 100% ethanol to give a final solution with a concentration of 200 μg/mL. Subsequent dilutions were performed using 100% ethanol to give standards with concentrations ranging from 0.049 – 25 μg/mL. All resulting solutions were vortexed to ensure thorough mixing and dissolution.

2.3. Stock solution and working standards of LEVO sodium in 0.1% w/v BSA

A stock solution of LEVO sodium was prepared by dissolving the appropriate mass of LEVO sodium in 0.1% w/w BSA solution to give a final solution with a concentration of 100 μg/mL. Subsequent dilutions were performed using 0.1% w/v BSA to give standards with concentrations ranging from 0.012 – 25 μg/mL. All resulting solutions were vortexed to ensure thorough mixing and dissolution.

2.4. Chromatographic conditions for LEVO quantification

Two methods for the separation and quantification of LEVO sodium were developed. The first (Method A; see Table 1) was carried out using RP-HPLC, on an Agilent 1220 Infinity II LC system (Agilent Technologies UK Ltd., Stockport, UK). The column used to achieve separation was a SphereClone™ C_{18} ODS (1) (95 Å pore size, 150 mm length x 4.6 mm internal diameter; 5 μm particle size) column Phenomenex, Cheshire, UK). The mobile phase used was composed of acetonitrile and 0.1% TFA at a ratio of 65:35% v/v, with a flow rate of 0.9 mL/min, injection volume of 50 μL, and a sample runtime of 10 min. UV detection of LEVO sodium was carried out at 225 nm.

The second (Method B; see Table 1) was carried out using RP-HPLC, on an Agilent 1220 Infinity II LC system. The column used to achieve separation was a Zorbax Eclipse plus C_{18} (95 Å pore size, 250 mm length x 4.6 mm internal diameter; 5 μm particle size) (Agilent Technologies UK Ltd., Stockport, UK) with guard column of matching chemistry. The mobile phase used was composed of acetonitrile and 0.1% TFA at a ratio of 50:50% v/v, with a flow rate of 0.6 mL/min, injection volume of 50 μL, and a sample runtime of 10 min. UV detection of LEVO sodium was carried out at 225 nm.

In each case, the mobile phase was degassed by sonication for 30 min prior to use. The column temperature was regulated to 30 °C. A needle wash using acetonitrile was carried out between each sample to ensure the injection needle was adequately clean. Chromatogram analysis was performed using Agilent Chemstation® software version B.02.01.

2.5. Method validation

Method validation for all compounds was carried out following the advice given in guidance in the ICH technical requirements for registration of pharmaceuticals for human use: Validation of analytical procedures Q2 (R1) 2005 [15]. The parameters assessed were specificity, accuracy, precision, linearity and range, LoD and LoQ.

To determine the specificity of the methods, blank samples were analysed, followed by analysis of a blank sample spiked with the compound of interest. In each analytical method, a minimum of three independent replicates were used to identify the analyte of interest from expected components and interferences.

The accuracy of each validated method was expressed in terms of mean percentage accuracy (± standard deviation (S.D.)). Accuracy was determined by analysing three samples of each compound with concentrations representing high, medium and low concentrations across the range. Intra-day (within 24 hr period) and inter-day accuracy (three consecutive 24 hr periods) were calculated. The percentage accuracy was determined using Equation 1.

$$\%\,accuracy = \frac{measured\,value}{true\,value} \times 100 \quad (1)$$

The precision of an analytical procedure was expressed as the variance, which utilises S.D. of the coefficient of variation (CV) of a series of measurements. In each validated method inter- and intra-day precision were assessed, with a minimum of three independent replicates indicated for low, medium, and high concentration sampling and expressed in terms of the percentage of CV. Percentage CV was determined using Equation 2.

$$\%\,CV = \frac{S.D.}{x} \times 100 \quad (2)$$

Where S.D. is standard deviation and x is the mean. Inter-day precision was calculated using one replicate of low, medium and high concentration from three 24 hr periods. Intra-day precision was calculated using thee replicates at low, medium and high concentration from one single 24 hr period.

To assess the linearity of each method, representative curves were plotted. Calibration curves were obtained over a period of three days and a minimum of five concentrations were tested across the range from three independent samples, prepared each day of the calibration. Mean values (±S.D.) were used to plot the final curves for each compound. Curves were analysed using least squares linear regression, determination of the slope, intercept, correlation coefficient (r^2) and residual sum of squares. The range was the highest and lowest concentrations tested which yielded a linear relationship and were representative of the samples likely to be analysed.

Table 1
Chromatographic conditions for the detection and quantification of LEVO sodium.

Method	Column and column temperature	Mobile phase composition	Flow rate (mL/min)	Injection volume (μL)	Detection Wavelength (nm)	Retention time (min)
A	SphereClone™ ODS (1) C_{18} (5 μm particle size, 4.6 x 150 mm) 30 °C	Acetonitrile: 0.1% TFA (65:35% v/v)	0.9	50	225	5.8
B	Zorbax Eclipse plus C_{18} (5 μm particle size, 4.6 x 250 mm) with guard column of matching chemistry 30 °C	Acetonitrile: 0.1% TFA (50:50% v/v)	0.6	50	225	6.6

An approach based on the S.D. of the response and the slope of the representative calibration curve was used to calculate LoD and LoQ (Equations 3 and 4, respectively).

$$LoD = \frac{3.3\sigma}{S} \quad (3)$$

$$LoQ = \frac{10\sigma}{S} \quad (4)$$

Where σ is defined as the S.D. of the output signal and S is defined by the slope of the linear regression model created as a result of correlation between known standard concentrations and the analytical procedure output.

2.6. Preparation of PCL implants containing LEVO

In order to prepare implants containing LEVO, moulds were created using silicone. For this purpose, a master mould was prepared using poly(lactic acid) (PLA) *via* 3D printing (Ultimaker 3; Ultimaker B.V., Geldermalsen, Netherlands) and subsequently a mixture of Silastic® S and a curing agent was poured into the 3D printed moulds and allowed to cure overnight. A representative image of these moulds can be seen in Fig. S1 (supporting information). The resulting silicone mould was used to prepare implants containing LEVO. A mixture containing 1.6 g of PCL and 0.4 g of LEVO was combined with 5 mL of DCM (5 mL). Subsequently this mixture was homogenised using a SpeedMixer™ DAC 150.1 FVZ-K (Hauschild & Co. KG, Hamm, Germany) (3,000 rpm for 20 seconds x 4). Finally, the mixture was cast into the silicone mould and DCM was allowed to evaporate. The resulting implants were removed from the moulds 2.5 x 40 mm when fully dried.

2.7. In vitro LEVO release study

LEVO release experiments were carried out using a solution containing 0.1% w/v BSA and 0.05% w/v of sodium azide (SA) (to prevent microbial growth) in deionised water as the release medium (pH 6.8 ± 0.1). The release experiments were carried out under sink conditions. Accordingly, LEVO concentrations were kept below 1/3 of its solubility during the release experiment. Each implant was placed in 50 mL of release medium (37 °C and 40 rpm). Every 3.5 days the release medium was replaced with fresh medium and the content of LEVO was analysed using the previously described HPLC method (Method B).

2.8. Statistical analysis

The mean, S.D. and CV were calculated where appropriate and a calibration plot was produced with least squares linear regression analysis and analysis of residuals. Where appropriate all data were expressed as a mean ± standard deviation (S.D.) and compared using one-way analysis of variance (ANOVA) with Tukey's HSD post-hoc. In all cases, $p < 0.05$ was the minimum value considered acceptable for rejection of the null hypothesis.

3. Results and discussion

There are a wide variety of drug delivery systems described in the literature including hydrogels, nano/microparticulate systems, implantable devices or more advance systems such as microneedles or micro-electro-mechanical devices [10,16–22]. These systems are being constantly refined to achieve sustained drug release. One of the key goals is to achieve drug delivery systems capable of providing a long-term effect whilst minimising drug uptake by the patient. Implantable devices are an attractive technology that can offer unattended drug release over prolonged periods ranging from months to years [10]. Drug stability is a crucial aspect in the development of these systems. Drugs loaded in long-acting drug delivery systems need to be stable within the system. Moreover, drug stability should be maintained during *in vitro* drug delivery experiments.

Hypothyroidism is a chronic condition that can be potentially treated with long-acting drug delivery systems. Companies such as Titan Pharmaceuticals are currently working on developing implantable devices for the long-term management of this condition [23]. LEVO is the treatment of choice to treat hypothyroidism [7]. However, the development of LEVO long-acting formulations is challenging as it is a highly unstable molecule in aqueous media [24]. In order to address LEVO stability issues, the use of a protein as stabiliser and the development of a suitable quantification method was described here.

3.1. HPLC method development. Method A

In order to evaluate LEVO stability in different solvents a RP-HPLC method was developed. The maximum absorption at 225 nm was chosen for analysis for this compound. Separation and quantification of LEVO sodium in 100% ethanol (Method A) was achieved using RP-HPLC. Following optimisation of the RP-HPLC method, the resultant chromatographic conditions yielded a sharp peak with an elution time of 5.8 min. Subsequently, the method was validated according to the ICH guidelines (see supporting information).

The short-term (24 hr) stability of LEVO sodium in a range of commonly used *in vitro* release study solvents was investigated (Fig. 1). LEVO stability in solution is a critical parameter for long-term release studies as the drug need to be stable in solutions for prolonged periods of time. LEVO sodium was found to be stable in ethanol (100%, 40% v/v and 20% v/v). In this work, stable was defined as retaining 90% of its original concentration. This criterion is widely accepted and is more strict than the criteria provided by ICH guidelines. ICH guidelines for the validation of bioanalytical methods specify that the stability of samples should be within a range of ±15% of the nominal concentration) [25].

The results obtained in ethanol and ethanol/water solutions were encouraging. However, these solutions are far from ideal as the high presence of ethanol will alter the release profile of LEVO due to its higher solubility in this solvent. Additionally, these release mediums are not representative of physiological conditions.

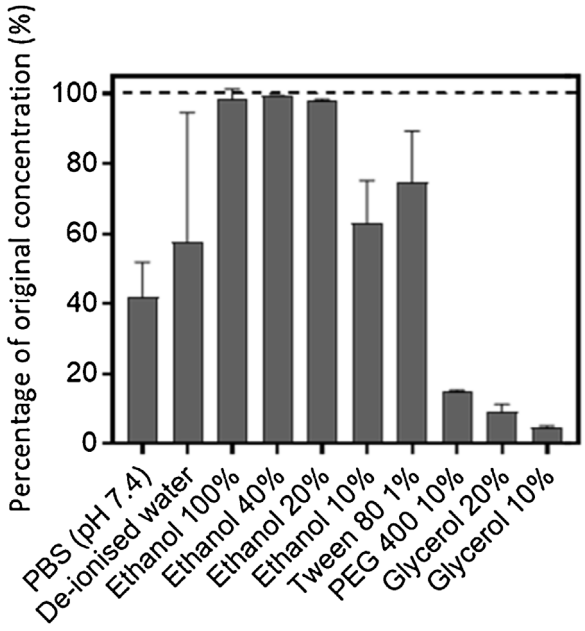

Fig. 1. Short-term (24 hr) stability of LEVO sodium in various solvents (means ± S.D., $n = 3$).

Therefore, there is a clear need of developing alternative stabilisers for LEVO.

The addition of other conventional stabilisers/solubilisers such as Glycerol, PEG or Tween 80 did not improve the stability of the drug. Indeed, some excipients such as PEG and glycerol contributed to increase the instability of LEVO (Fig. 1).

In the body, LEVO circulate bound to thyroid binding globulin, human serum albumin and transthyretin [26]. LEVO is highly bound to plasma proteins (99% protein binding) [8]. LEVO is reported to have a half-life of seven days in the blood stream [8]. Therefore, it was hypothesised in this article that protein binding in the blood stream could contribute to the increased stability observed for circulating LEVO sodium. It has been reported that approximately 76% similarity has been found in bovine serum albumin compared to human serum. Additionally, in some physical characteristics, these albumins could not be distinguished [27]. Moreover, BSA is more affordable than human serum albumin. Therefore, BSA was investigated as a potential stability enhancer for LEVO sodium *in vitro* release studies.

Interestingly, due to its high protein binding the HPLC method previously developed could not be used for LEVO quantification. BSA and LEVO were bound together and LEVO peak could not be separated from the BSA peak. Consequently, a new HPLC method was developed.

Table 2
Determination of accuracy of RP-HPLC for the detection and quantification of LEVO sodium in 0.1% w/v BSA (means ± S.D., n=3).

Theoretical concentration (μg/mL)	Intra-day accuracy Mean accuracy ± S.D. (%)	Inter-day accuracy Mean accuracy ± S.D. (%)
0.78	100.68 ± 4.10	99.09 ± 8.32
6.25	101.08 ± 5.33	103.53 ± 3.72
25	104.36 ± 4.61	103.60 ± 8.02

3.2. HPLC Method validation for LEVO sodium in 0.1% w/v BSA. Method B

A new HPLC method was developed to quantify LEVO in solution in the presence of BSA. As the method will be developed for long-term drug release experiments, SA was added (0.05%) to prevent bacterial growth in the release media [10].

The column used was a C_{18} column. However, the main difference with the previously used one was the column length. Using a longer column allowed LEVO separation from BSA (Fig. 2). Therefore, an HPLC method was developed and validated following ICH guidelines. First, specificity was determined by running a blank sample (0.1% w/v BSA or 0.1% w/v BSA with SA) and a blank spiked with LEVO sodium. Fig. 2 shows representative traces of the samples. A retention time of 6.6 min was observed and the peak of interest was distinct from any interfering compounds. LEVO sodium has poor stability in many commonly used aqueous media (including PBS (pH 7.4) and water), therefore, a suitable aqueous release medium for *in vitro* release studies was required. The presence of BSA was found to stabilise LEVO sodium in solution and thus can be used at the *in vitro* release medium. Method B was tested using a Zorbax Eclipse plus C_{18} column with the samples in 0.1% w/v BSA, however, a peak which had considerable fronting and was not fully resolved from the BSA peak resulted. The proportion of aqueous phase in the mobile phase was increased to 50% and the flow rate was reduced to 0.6 mL/min, both of which served to reduce peak fronting and increase resolution of the peaks. Reduction of the flow rate increased the retention time; however, it also increased the peak areas and, therefore, the sensitivity of the method. These conditions resulted in a well resolved sharp peak with a retention time of 6.6 min.

The next step was to determine accuracy. This parameter was determined in triplicate, over three concentrations which are representative of the range seen in the calibration curve. Measured concentrations were compared to theoretical value and percentage accuracy was calculated and shown in Table 2. In all cases, percentage accuracy was found to be in the range of 99 – 105%.

Precision was analysed with respect to repeatability over short time periods (intra-day) and intermediate time periods (inter-day).

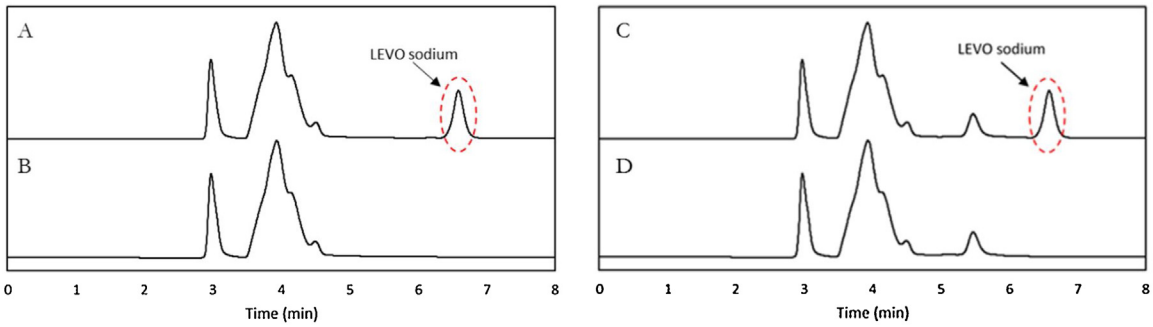

Fig. 2. Representative RP-HPLC chromatograms of (A) LEVO sodium (50 μg/mL) in 0.1% w/v BSA and (B) 0.1% w/v BSA. (C) LEVO sodium (50 μg/mL) in 0.1% BSA with SA (0.05%) (D) 0.1% w/v BSA with SA (0.05%).

Table 3
Inter-day and intra-day precision of the detection and quantification of LEVO sodium in 0.1% w/v BSA (means ± S.D., $n = 3$).

	Intra-day precision		Inter-day precision	
Theoretical concentration (μg/mL)	Mean concentration ± S.D. (μg/mL)	CV (%)	Mean concentration ± S.D. (μg/mL)	CV (%)
0.78	0.79 ± 0.03	4.08	0.76 ± 0.06	7.29
6.25	6.32 ± 0.33	5.27	6.74 ± 0.44	6.56
25	26.29 ± 1.34	5.07	24.77 ± 2.08	8.40

Table 4
Calibration curve properties for LEVO sodium in 0.1% w/v BSA (means, $n = 9$).

Concentration range (μg/mL)	r^2	y-intercept	Slope	LoD (μg/mL)	LoQ (μg/mL)
0.012 – 25	1.0	17.94	108.18	0.03	0.09

Intra- and inter- day precision are presented as CV for LEVO sodium at high, medium and low concentrations in Table 3. CV was calculated as an indicator of precision, and in all cases was found to be less than 9%.

The ICH guidelines do not specify acceptance criteria for accuracy and precision, however, González et al. [28] recommend that percentage deviation from the true value and percentage CV should not exceed 15% (or 20% for samples with lower concentrations) for precision and accuracy, respectively. Therefore, the present method is compliant with these recommendations.

To assess linearity of the method, a range of concentrations was analysed. Samples were prepared in triplicate and repeated on three consecutive days. Mean values (±S.D.) were used to produce a final representative calibration plot. Calibration curve properties are shown in Table 4. Interestingly the concentration range is wider than the ranges described by other HPLC methods described in the literature for *in vitro* LEVO testing using UV-visible detection [7]. Obviously, there are methods with lower limits of detection/quantification using mass spectrometry detection or not conventional detectors. However, these methods are developed to quantify LEVO within biological matrices [29,30].

This analytical method was validated according to the ICH guidelines [15]. This provides a high degree of assurance that the method is suitable for its intended purpose and promotes continuity and transferability to the process of validation [31]. ICH guidelines do not specify acceptance criteria, but do recommend the parameters that should be considered [15].

3.3. LEVO Stability – long-term

The HPLC method described in the previous section was used to evaluate if BSA was effectively increasing the stability of LEVO. Fig. 3A shows the stability of LEVO in two different BSA containing solutions. It can be seen that 0.1% w/v of BSA was enough to stabilise the drug over a period of 24 h. A lower BSA concentration was also tested (0.05% w/v). However, it was obvious that this concentration was not enough to guarantee LEVO stability over 24 h. Therefore, 0.1% w/v BSA was used for subsequent tests.

Extended stability studies were conducted with the most promising solvent from the short-term stability data (section 3.1). BSA was chosen over ethanol because an aqueous solution will give more biologically relevant *in vitro* results. LEVO sodium was found to be stable in 0.1% BSA w/v for at least 14 days (retaining over 90% of its original concentration) at all concentrations tested (50 μg/mL, 12.5 μg/mL and 3.125 μg/mL) and under all conditions tested (room temperature, fridge and incubator) (Fig. 3B-3D). Interestingly, SA was added to the release medium at a concentration of 0.05% w/v to prevent microbial growth and was not found to have an adverse effect on the stability of LEVO sodium. LEVO sodium was not stable in 0.05% w/v BSA for 24 hr and, therefore, it was concluded that the stability enhancement of BSA was concentration dependent. LEVO sodium was found to be stable in 0.1% w/v BSA up to 50 μg/mL for up to 14 days.

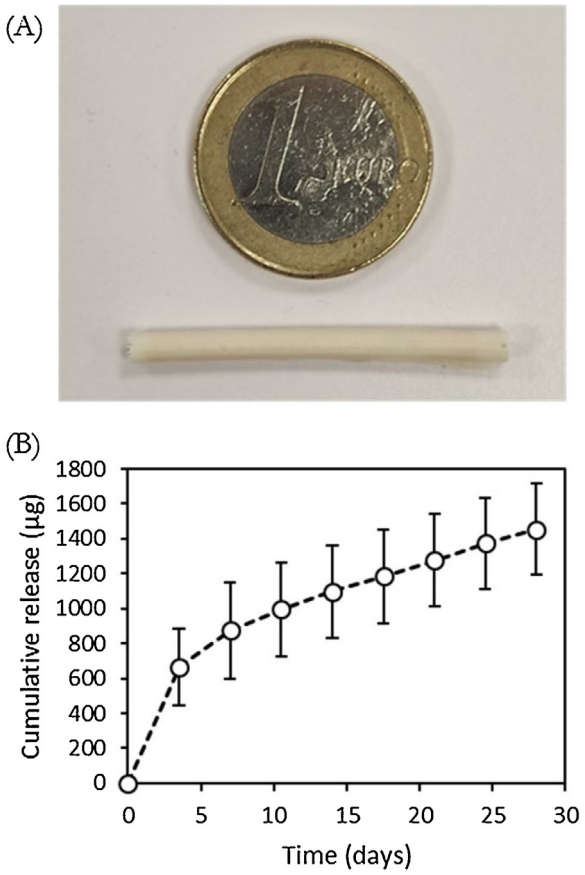

Fig. 4. Photograph of the implant next to a 1€ coin (A). LEVO release from the PCL-based implant over a period of 28 days (B).

3.4. Levothyroxine release from a PCL-based implant

The HPLC method described in previous sections was tested by evaluating the release of LEVO from implantable devices designed for prolonged drug delivery. The dimensions of these implants, 2.5 x 40 mm (see Fig. 4A), are in line with previously described implants (Stewart, Domínguez-Robles, McIlorum, Gonzalez, et al., 2020; Stewart, Domínguez-Robles, McIlorum, Mancuso, et al., 2020).

They contained 20% (w/w) of LEVO and 80% (w/w) of PCL. PCL is a biocompatible and biodegradable material that has been extensively used for drug delivery applications [32]. Therefore, it is an ideal candidate for this purpose. The release of LEVO from these devices was evaluated using the HPLC method described before and BSA as stabiliser for a period of 30 days (Fig. 4B). The device provided an initial burst release after the first 3 days and a subsequent linear release over the following days. After 28 days the implant released 4.7 ± 0.7 % of the initial LEVO loading. Moreover, the daily

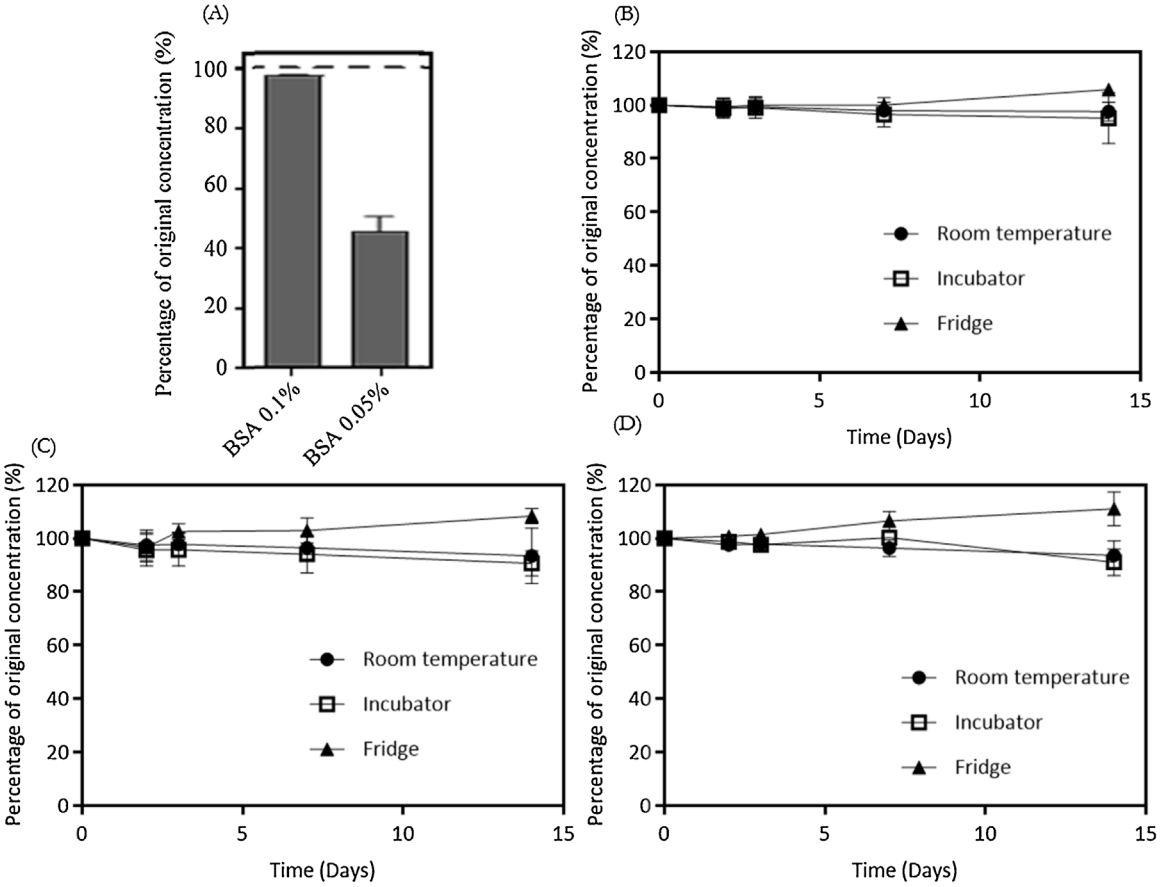

Fig. 3. (A) Stability of LEVO in BSA solution over 24 h. Stability of LEVO sodium in 0.1% w/v BSA (B) 50 μg/mL; (C) 12.5 μg/mL; (D) 3.125 μg/mL (means ± S.D., n = 3).

dose released during this linear portion was around 30 μg/day. The daily dose of this compound range between 25 and 200 μg/day [33]. Based on the obtained results this implant should be able to cover the daily dose of patients requiring the lowest dose. However, in order to be used to treat patients requiring higher doses more than one implant will be required. The use of multiple implants to provide adequate drug dosing to patients has been described before [34]. A good example of this is Norplant a contraceptive implant that consist of 6 implants capable of providing sustained release of levonorgestrel for up to 5 years [34]. The initial burst release can be problematic for the patient if more than a single implant is required. In this case the implants can be initially washed in ethanol to remove the LEVO prior to the implantation of the device.

In order to evaluate the amount of LEVO released from long-acting formulations *in vitro* the drug need to be stabilised for at least 24 h to allow medium replacement. It has been proven that LEVO is highly unstable unless BSA is added to the release media to stabilise the drug. In order to develop LEVO long-acting formulations it is vital to have reliable *in vitro* methodologies. Therefore, the combination of BSA as stabiliser and an HPLC method to quantify the mount of drug in solution is a good approach to address this issue.

4. Conclusions

Working with LEVO has been demonstrated to be challenging due to its instability issues. There are quantification methods described in the literature to quantify the dissolution of this drug from conventional solid oral dosage forms [7]. For these types of formulations, the stability issues are not a limiting factor as the dissolution experiment last less than one hour. However, there is a growing interest in long-acting drug delivery systems such as long-acting injections or implantable devices in order to improve medication adherence. The development of such systems requires reliable *in vitro* quantification methodologies. LEVO stability in solution will start to decline after a few hours (less than 7 h) [24]. Therefore, stabilising compounds will be required in the release/dissolution media to overcome this limitation for long-acting drug delivery systems. The use of BSA was proposed in this work as it was shown to provide extended stability to this drug over periods of up to 14 days. Additionally, due to LEVO's high protein binding a modified HPLC method was required. Finally, the release of LEVO from polymeric implants was evaluated using the described method for up to 28 days. The combination of a new HPLC method and the use of BSA could be potentially used for the development of new long-acting LEVO drug delivery systems.

CRediT authorship contribution statement

Sarah A. Stewart: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **David Waite:** Methodology. **Juan Domínguez-Robles:** Supervision, Writing - review & editing. **Emma McAlister:** Methodology. **Andi Dian Permana:** Writing - review & editing. **Ryan F. Donnelly:** Supervision, Writing - review & editing. **Eneko Larrañeta:** Supervision, Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2021.114182.

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