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Characterization of a potential medium for 'biorelevant' in vitro release testing of a naltrexone implant, employing a validated stability-indicating HPLC method

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Abstract

A variety of factors have been recognized that influence media optimization for drug release studies of implant dosage forms. Of primary importance is selection of a medium that physiologically mimics the milieu at the site of administration (a condition termed 'biorelevance'). We describe in this paper, the characterization of Hanks' balanced salts solution, with necessary modification, for application as a 'biorelevant' medium for in vitro release studies of a biodegradable, subcutaneous implant of naltrexone. A detailed investigation of changes in pH, osmolality and ultraviolet (UV) spectrum as a function of time and temperature was conducted. Variation in the parameters evaluated was found to be within acceptable limits. Validation of a simple and selective, high performance liquid chromatography (HPLC) assay method for naltrexone was carried out to evaluate stability. The calibration curves were linear from 0.16 to 20.00 $\mu g\,ml^{-1}$. Imprecision and inaccuracy were less than 2% and no interference was observed from degradation peaks. Stability studies of naltrexone indicated the media should be replaced every 7–8 days for real-time testing. This was applied to an investigation of in vitro drug release. The method has been proven to be suitable for investigation of naltrexone released from the implant.

Keywords: Modified Hanks' balanced salts solution; Naltrexone; Implant; HPLC assay

1. Introduction

The identification of appropriate media for in vitro studies of dosage forms is critical to the assessment of drug release [1]. Corrigan et al. [2] observed that the sensitivity of in vitro–in vivo relationships (IVIVR) to medium composition could be attributed to systematic shifts in release profiles as a function of changes in pH and buffer capacity. These workers also reported that difficulties exist in making a rational choice for a suitable dissolution medium.

In an earlier article, we reviewed method development strategies for profiling in vitro drug release from subcutaneous implant dosage forms [3]. Among various factors listed, an understanding of physiological variables at the site of implantation was given priority for the design of drug release tests that provide more clinically meaningful specifications. Also, the potential for use of buffers that would mimic physiological condition in vivo was recognized. An assessment of the literature, however, revealed that most in vitro release methods to study implants did not utilize 'biorelevant' media [4–6]. In support of this underlying need, it was decided to employ the Hanks' balanced salts solution (HBSS), with suitable modification, for the present in vitro release study of a naltrexone implant dosage form.

In this endeavor, it is important to consider the properties desired of an ideal drug release medium [3]. Extracellular interstitial fluid differs from plasma because it contains fewer protein ions, primarily due to the impermeable character of a normal capillary membrane. Also, Na^+ and Cl^- form the most abundant ions in extracellular fluid [7]. The role of Hank's balanced salts solution has been well documented in several papers on maintenance of tissue viability [8–11]. Hanks established the formula for this solution in 1948, an application for which was shown

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in tissue culture experiments the following year [12,13]. The components (in $g\,l^{-1}$) included: $CaCl_2{\cdot}2H_2O$, 0.185; $MgSO_4$ (anhyd.), 0.09767; KCl, 0.4; KH_2PO_4 (anhyd.), 0.06; NaCl, 8.0; Na_2HPO_4, 0.04788; glucose, 1.0; $NaHCO_3$, 0.35; Phenol Red, 0.011. The phosphate and bicarbonate equilibria are responsible for maintenance of the pH in the physiological range (~7.4). Primary considerations for selection of HBSS as the release medium of choice for the present study were: (a) the simplicity of the formula and well defined components, (b) a comparable inorganic phosphate concentration to the human plasma level [14,15] and (c), adequate solubility of naltrexone [11] to ensure 'sink' conditions throughout the period of study. Modifications to the medium composition were incorporated, as detailed in Section 2, and the medium has been subsequently referred to as "modified Hanks' balanced salts solution".

Naltrexone, an opiate receptor antagonist, has been identified as a candidate for formulation as an implant to overcome patient non-compliance to dosage schedules of the tablet [16]. Various research groups are currently investigating development of dosage forms, such as implants and depots, to provide a long-acting alternative to the tablet [17–19]. Trissel reports the stability for a compounded oral product of naltrexone [20]. The analytical method employed for that evaluation had been developed by Fawcett et al. [21], comprising a high performance liquid chromatography (HPLC) procedure for detection of naltrexone at an absorbance detector wavelength of 214 nm. The compendial monograph cites a reversed-phase HPLC assay for naltrexone hydrochloride powder and tablets [22]. Other workers have employed method modifications to HPLC assays to suit specific needs [11,23,24]. A selective and sensitive method is not available however, for quantification of naltrexone in modified Hanks' balanced salts solution as employed in our study.

This is the first publication that reports characterization of a new medium for implant release studies. A description of pH, osmolality, buffer capacity and spectral changes for modified Hanks' balanced salts solution for its suitability as a 'biorelevant' medium is included. The dosage form was a biodegradable, subcutaneous implant of naltrexone. Three temperature levels, viz. 38 °C (canine body temperature), 45 °C (an intermediate level) and 55 °C (determined by the melting point range of the polymeric implant matrix, 59–64 °C), besides 25 °C (room temperature) have been targeted for media characterization. Real-time (38 °C) drug release assessment is a basic requirement for in vitro characterization. The elevated temperatures of 45 and 55 °C were evaluated to accommodate potential accelerated in vitro studies. An accelerated test would involve a departure from 'biorelevance' in terms of the parameter used to induce acceleration in the release rate, in this case, elevated temperature. 'Biorelevance' is therefore not entirely maintained at the elevated temperatures.

2. Experimental

2.1. Chemicals and reagents

Naltrexone hydrochloride (working standard) was obtained from Sigma (St. Louis, MO, USA). Fig. 1 shows the structure of naltrexone. Hanks' balanced salts, 4-(2-hydroxy ethyl) piperazine-1-ethanesulfonic acid (HEPES buffer, 1 mM) and analytical grades of triethylamine and orthophosphoric acid were purchased from Sigma. Primocin was procured from InvivoGen (CA, USA). Potassium phosphate (monobasic, anhydrous) and sodium hydroxide were purchased from Fisher Scientific (Fairlawn, NJ, USA). Acetonitrile (HPLC grade) was purchased from Burdick & Jackson (Honeywell International Inc., MI, USA). Water was obtained in-house using the Nanopure Diamond water system (Barnstead International, IO, USA).

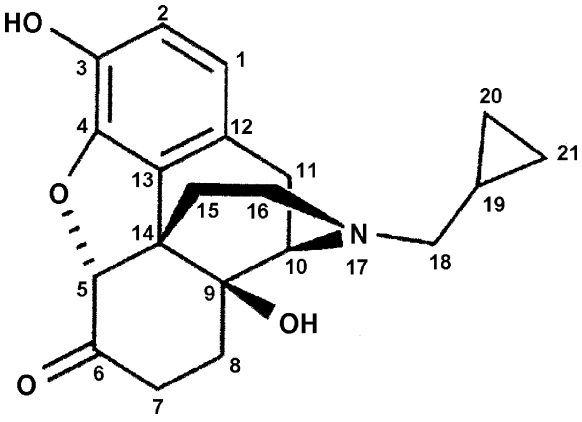

Fig. 1. Structure of naltrexone.

2.2. Description of the modified Hanks' balanced salts solution

A modification to the formula of Hanks' balanced salts involved deletion of two components: Phenol Red and sodium bicarbonate. Phenol Red is an indicator that potentially interferes in chromatographic separations [25]. Sodium carbonate provided additional buffer capacity through a continuous bubbling of CO_2, such that the O_2/CO_2 exchange processes in living tissue cells could be simulated. Since purge gas was not a viable option during this study, and because no cell culture was involved, we chose to employ 4-(2-hydroxy ethyl) piperazine-1-ethanesulfonic acid (HEPES) as an enhancer of buffer capacity [26]. A higher buffer capacity could be anticipated as a necessity because a future objective of the project was to investigate drug release under accelerated conditions. HEPES has a pK_a of 7.3 at 37 °C, and is commonly used to arrest the drift in pH of physiological media [27,28]. In addition, since the intention was to use the medium for at least 1 month, it became essential to incorporate an anti-microbial agent to its formula. Most common antibiotics have a shelf life not greater than one week at mammalian body temperature [29]. Therefore, a new antimicrobial agent, Primocin (0.2%, v/v), was identified. Primocin has a broad-spectrum of activity against Gram (+) and Gram (−) bacteria, fungi and mycoplasma, and is stable for at least 1 month at 37 °C [30]. The addition of HEPES and Primocin are common in cell culture media and do not affect the viability of cells, thus being consistent with the criteria of 'biorelevance'.

The following procedure was adopted for media preparation: 9.8 g of the commercially available modified Hanks' balanced

salts was accurately weighed and dissolved in 975 ml of water. The resulting pH of 6.6 ± 0.2 was adjusted to 7.4 ± 0.1 with sodium hydroxide (10 mM) in water. Twenty-five milliliters of HEPES solution (1 M) was then added, followed by 2 ml of Primocin. The solution was mixed throroughly and filtered through a 0.45 μm filter. Finally, the pH was again adjusted to 7.40 ± 0.05, if required, at the designated study temperature.

2.3. Medium characterization

2.3.1. pH changes as a function of temperature

Changes in pH were monitored using a pH meter (Model 340; Corning Instruments, NY, USA) equipped with probe for temperature correction. Two hundred milliliters of medium in a stoppered flask was placed on a magnetic stirrer with temperature control. Continuous stirring at slow speed and a stepwise gradient increase of 1 °C were employed. Temperature measurements were made with a thermocouple thermometer (Model 600-1040; Barnant Company, IL, USA) having a least count of 0.1 °C. A time of 5 min was fixed for equilibration at each step before the stirring was stopped, and the pH recorded immediately. Stirring influences pH measurements, at times, by affecting any existing junction potential adversely [31]. The pH meter was also re-calibrated at each temperature level with standard buffers maintained at the same temperature. This ensured that the pH drift of the electrode had been corrected [31]. Recordings of pH were made between 24 and 56 °C. When the highest temperature was attained, to crosscheck the readings, a downside gradient of 1 °C step size was made to 24 °C, while the pH was recorded as before. The complete procedure was repeated in triplicate to ensure reproducibility. An inert gas atmosphere was not used in the headspace of the flask. This was because the purpose of the study was to simulate real-time drug release under conditions in which atmospheric carbon dioxide and oxygen are expected to play a role.

2.3.2. Buffer capacity changes as a function of temperature

An incremental addition of sodium hydroxide solution (10 μl, 10 mM) was made to 200 ml of the medium, maintained at a fixed temperature. pH determinations were carried out at the temperatures, 38, 45 and 55 °C as mentioned earlier, along with an assessment at room temperature. Also, the efficiency of HEPES was evaluated by testing HBSS alone.

2.3.3. Ultraviolet spectrum changes as a function of temperature and time

Absorption changes were evaluated using a UV/vis spectrophotometer (Model Lambda 2S; Perkin-Elmer Instruments, MA, USA) in the wavelength range of 190–300 nm. Any potential interfering contamination due to naltrexone sticking to cuvettes was avoided by thorough rinses of water and methanol between consecutive measurements. A spectrum of the modified Hanks' balanced salts solution was recorded on the day medium was prepared (day 0), and compared to spectra obtained on days 15, 30, 60 and 90 post-storage of aliquots maintained at room temperature, 38, 45 and 55 °C. Prior to spectral evaluation, the aliquots were cooled to room temperature to prevent errors arising out of changes in absorptivity. A freshly prepared medium was treated each time as a reference along with test media to check for any baseline drift.

2.3.4. Osmolality changes as function of temperature and time

Osmolality studies were conducted using an Osmometer (Model 3W; Advanced Instruments Inc., MA, USA) based on depression in freezing point. The meter was calibrated at 100 and 900 mOsm using standards supplied by the vendor. Care was taken to set the temperature of standards and test solutions at the same values prior to testing. A 2.0 ml aliquot of solution was analyzed each time. Osmolality was recorded on the day medium was prepared (day 0), and on days 7, 15, 30, 60 and 90 for aliquots stored at room temperature, 38, 45 and 55 °C. On each of the days, a freshly prepared medium was also tested to check for any instrument variations from day 0.

2.4. Validation of the HPLC analytical method

A stability-indicating HPLC method was developed and validated for analyzing the in vitro samples. It employed UV detection and no sample preparation was involved.

2.4.1. Preparation of stock solution

Approximately 10 mg of naltrexone hydrochloride working standard was accurately weighed and transferred into a 10 ml volumetric flask. It was dissolved in methanol:water (50:50, v/v) to yield a stock solution, the exact concentration of which was corrected for naltrexone freebase. The stock solution was stored below 10 °C.

2.4.2. Preparation of standards and quality control samples

From the stock solution, a serial dilution was performed in modified Hanks' balanced salts solution to yield calibration curve standards ranging from 0.16 to 20.0 μg ml^{-1}. Similarly, quality control samples were prepared at 0.45, 4.0, 8.0 and 18.0 μg ml^{-1} representing low (LQC), middle (MQC-1 and MQC-2) and high (HQC) controls, respectively. Aliquots were stored in capped polypropylene tubes below 20 °C until analysis.

2.4.3. Sample preparation

No sample filtration or extraction was required. The aliquots were thawed at room temperature, and transferred into 2 ml glass HPLC vials for analysis.

2.4.4. Chromatography

A modification to the method of Fawcett et al. was carried out [21]. The Shimadzu VP LC system consisted of a system controller (SCL-10A), a high-pressure pump (SIL-10AD), an autosampler (SIL-10AD) and a diode-array detector (SPD-M10A). The chromatographic separation was achieved using a Supelcosil C_{18} column (5 μm; 4.6 mm × 150 mm, Supelco Corporation, PA, USA) maintained at 50 °C in a column oven (CTO-10AC). The mobile phase was prepared premixed. Eighty-eight parts (by volume) of a solution containing monobasic potassium

phosphate (40 mM), triethylamine (0.06%, v/v) and orthophosphoric acid (for pH adjustment to 4.75) in water was mixed with 12 parts (by volume) of acetonitrile. An isocratic flow rate of 1.25 ml min^{-1} was found optimal. Acetonitrile:water (50:50, v/v) was used as a rinse solution for the injector, and the injection volume was fixed at 5 μl. Detection was carried out using a wavelength of 204 nm. Also, the peak purity for naltrexone was determined.

2.4.5. Forced degradation

It is essential that the incorporated drug must have sufficient stability in medium for either evaluation of real-time or accelerated release from a dosage form. This is because drug would accumulate over a period of time in media reservoirs. For implants employed in this study, this time was estimated, based on preliminary data from a prototype, to be of the order of a few months. Therefore, a forced degradation experiment of naltrexone in modified Hanks' balanced salts solution was necessary. Based on this investigation, it is possible to stipulate a time interval before complete media replacement should be conducted during release testing at any given temperature. This procedure enables further fresh drug released from the dosage form to accumulate in the reservoir.

2.4.5.1. Solution preparation and storage condition. From the stock solution of naltrexone, dilutions were prepared at 0.45, 4, 8 and 20 μg ml^{-1} to cover the entire dynamic range of the method. Aliquots of all solutions were stored in capped borosilicate glass vials (to simulate the reservoir material used for actual studies) at room temperature, 38, 45 and 55 °C. An analysis of aliquots after 2, 4, 8 and 16 days was conducted by HPLC, and naltrexone concentrations at each level were compared to day 0 values.

2.4.5.2. Qualitative LC–MS support. To identify peaks of potential degradation products observed through HPLC, a Waters Micromass Quattro mass spectrometer (Waters Corporation, Manchester, UK) with Mass Lynx ver. 3.4 software for data acquisition was used. A high-pressure pump (Shimadzu DGU-14A), a system controller (Shimadzu SCL10ADVP) and an HTS-PAL autosampler (Carrboro, NC, USA) was configured with the mass spectrometer (MS). Since the HPLC–UV analysis employed phosphate buffer and a high mobile phase flow rate, this mobile phase could not be used for mass spectroscopy. Therefore, a mobile phase comprised of water with ammonium acetate (20 mM):acetonitrile (20:80, v/v) was employed. A soft, electrospray ionization (ESI) technique in the positive ion mode was used, and a moderate optimization of response for naltrexone in the Q1 mode was carried out by an infusion of a 1 μg ml^{-1} solution in methanol. The following values were obtained and set for the analysis—capillary 3.5 kV; cone 40 V; extractor 3 V; source temperature 150 °C; ESI probe temperature 300 °C. Nitrogen was used as the sheath gas at 300 l h^{-1}. Once the system had been optimized, a scan range of *m/z* 100–1000 was fixed for sample analyses. The same analytical column at a flow rate of 0.25 ml min^{-1} was employed for both HPLC and MS to check for corresponding retention times of drug and degradation products.

Table 1
Buffer capacity (β) of medium under different conditions

Condition (°C)	β
25	9.1×10^{-4}
38	9.8×10^{-4}
45	7.7×10^{-4}
55	7.1×10^{-4}
25 (HBSS alone)	2.4×10^{-5}

3. Results and discussion

In this study, a modified Hanks' balanced salts solution was characterized for various parameters, including pH, buffer capacity, osmolality, spectra and stability of naltrexone in the medium. Stability testing was conducted with a validated HPLC procedure to establish the time interval before complete media replacement during release testing studies.

3.1. Media characterization

3.1.1. pH and buffer capacity

The pH was found not to vary beyond 0.02 between repeated measurements ($n = 3$) at any given temperature. There was a decrease in pH with an increase in temperature, and a corresponding increase in pH when the temperature was lowered back to 24 °C. This was justified thermodynamically from the positive value of enthalpy change ($\Delta H = +3.6$ kJ mol^{-1}) for the phosphate equilibrium, based on LeChatelier's principle [32]. For every 1 °C, the observed pH decreased by 0.015, which concurs with the published value of 0.014 [33]. This decrease was best described by a second-order polynomial fit as: pH = (7.52E−05[temperature (°C) − 40.94]^2) − (1.46E−02[temperature (°C)] + 7.724), with an r^2 of 0.99. The pH changes at elevated temperatures necessitated addition of different amounts of sodium hydroxide for maintaining the medium within the physiological range. This could in turn have an influence on osmolality changes, a description of which is provided in the following section.

The Van Slyke equation was used for calculation of buffer capacity, and the values are presented in Table 1. An addition of HEPES to Hanks' balanced salts solution resulted in a 38-fold increase in buffer capacity at room temperature. The variation observed following an increase in temperature from 38 to 55 °C was relatively small (1.36-fold decrease).

3.1.2. Spectral evaluation

The changes in UV absorption of the medium as a function of temperature and time are better understood when the day 90 spectra collected are represented overlaid (Fig. 2a). Fig. 2b is a spectrum of the Hanks' balanced salts solution with HEPES added, but without Primocin. Clearly, the contribution of the antimicrobial agent in the UV absorption spectrum can be observed. Also, the spectrum of the medium stored at 55 °C

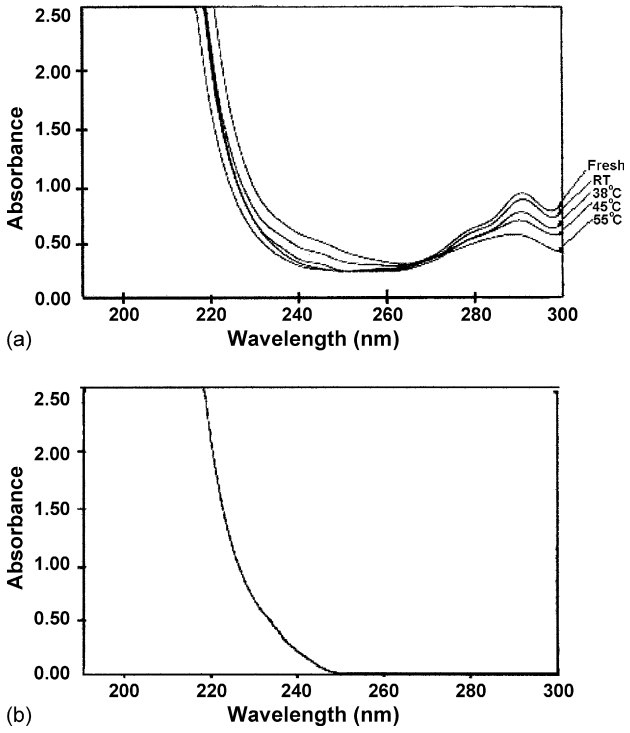

Fig. 2. (a) Absorption spectra of the modified Hank's balanced salts solution at day 90, with the spectrum of freshly prepared solution overlaid for comparison and (b) absorption spectrum of the release medium with out added antimicrobial agent.

is distinctly different from that obtained for the medium maintained at 38 °C. Although there is no difference in absorption up to approximately 260 nm, absorption due to the antimicrobial agent seems to have decreased, representing a possible degradation of its constituents. The additional moieties could result in a change in colligative properties of the medium, the most pertinent being a variation in osmolality. This further authenticated the study of osmolality as a required characteristic for suitability of the medium.

3.1.3. Osmolality

The previously described experiments indicated a degradation of components that could potentially cause the medium to exceed the normal iso-osmolar range (270–330 mOsm [9,14]). Table 2a summarizes osmolality values of the medium as a function of temperature and time following the day of preparation (day 0). In Table 2b, the osmolalities of a freshly prepared Hanks' balanced salts solution without added HEPES, HEPES in water (25 mM) and Primocin in water (0.2%, v/v) are provided for comparison.

Table 2a
Osmolality changes[a] of medium as a function of temperature and time

Day	Condition		
	38 °C	45 °C	55 °C
7	282.3	284.7	286.2
15	282.7	289.1	291.0
30	284.9	292.3	301.8
60	285.4	303.4	308.9
90	288.1	305.2	321.6

[a] Values within a R.S.D. of 1% ($n = 5$).

Table 2b
Contribution of the individual components in a freshly prepared medium

Component description	Osmolality (mOsm)
HBSS	267.1
HEPES (25 mM)	14.8
Primocin (0.2%, v/v)	–[a]
Total	281.9
Total osmolality (observed)	281.3

[a] Dip in osmometer readings observed indicating negligible values.

Although the difference in osmolality was significant ($p < 0.05$) at day 90, values were within the normal physiological range. This provided further substantiating data for physiological relevance of the medium.

3.2. HPLC method validation

The system suitability parameters were found to be within acceptable limits (Fig. 3). An analytical run time of 8 min was optimized for each sample because of elution of a peak at 11 min. This peak was later attributed to a medium component.

3.2.1. Linearity

The calibration curves were linear in the range of 0.16–20 μg ml^{-1} ($r^2 > 0.99$). The data are presented in Table 3. The concentration residuals ranged from −1.9 to 2.7% (R.S.D. = 1.9–4.8%; $n = 6$). Fig. 4 shows representative chromatograms of blank medium, the lowest standard and a QC sample. The LLOQ of the method, 0.16 μg ml^{-1}, was calculated by injection of samples prepared independent of the calibration curve from its slope. The deviation was found to be 1.4% of the nominal concentration (R.S.D. = 1.7%; $n = 6$).

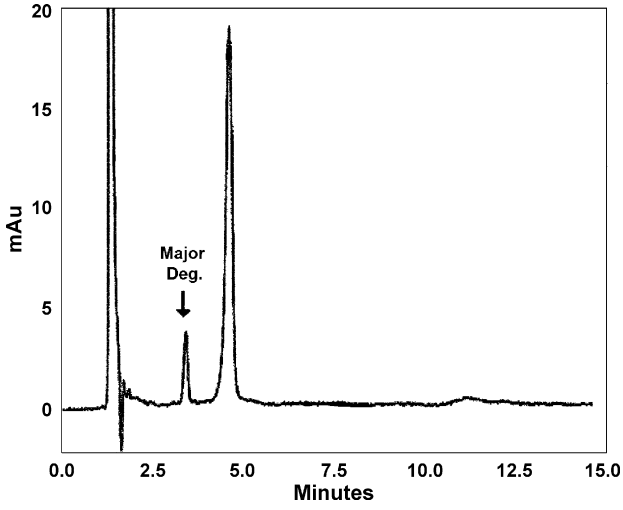

Fig. 3. A chromatogram of a degraded sample to show resolution of naltrexone from its degradation product. The analysis time was optimized during validation to 8 min to avoid carryover of the peak at 11 min to subsequent injections. System suitability parameters—capacity factor (k'), 1.5; tailing (T), 0.83; theoretical plates (N), 2452; separation factor (α), 1.6; resolution (R), 2.1.

Table 3
Reverse predicted concentration residuals of naltrexone

Set number	Nominal concentration (μg ml^{-1})								r^2	Slope	Intercept
	0.16	0.31	0.63	1.25	2.50	5.00	10.00	20.00			
1	0.16	0.30	0.62	1.33	2.54	4.64	9.10	21.28	0.9929	15869	−0.074
2	0.17	0.30	0.62	1.20	2.57	5.03	10.05	19.91	0.9999	15378	0.102
3	0.16	0.31	0.58	1.17	2.43	4.85	9.87	20.46	0.9992	16353	0.060
Mean	0.16	0.30	0.61	1.23	2.52	4.84	9.67	20.55		15867[a]	0.03[b]
S.D.	0.00	0.01	0.02	0.09	0.07	0.20	0.51	0.69		487	
%R.S.D.	2.2	2.9	4.1	7.1	2.9	4.1	5.2	3.3		3.1	
%DFN	2.5	−2.3	−3.6	−1.4	0.6	−3.3	−3.3	2.7			

[a] C.I. (14656, 17078).
[b] Not significantly different from zero ($p > 0.05$).

3.2.2. Precision and accuracy

The precision and accuracy of the method were reported as the relative standard deviation (%R.S.D.) and percent deviation from the nominal value (%DFN). The global inter-day precision and accuracy for three batches ($n = 9$) were found to be between 1.4 and 1.7%, and −1.0 and 0.1%, respectively (refer to Table 4). These values are well within acceptance criteria [34,35].

3.2.3. Autosampler stability

Autosampler stability was validated by a comparison of the peak areas of QC samples injected at 4 and 24 h to the mean peak areas of samples obtained at 0 h. The mean values of autosampler stability after 4 and 24 h were 100.3–106.7% (R.S.D. = 1.2–3.4%) and 100.1–102.2% (R.S.D. = 0.3–1.2%), respectively, of the initial QC concentrations measured at time 0.

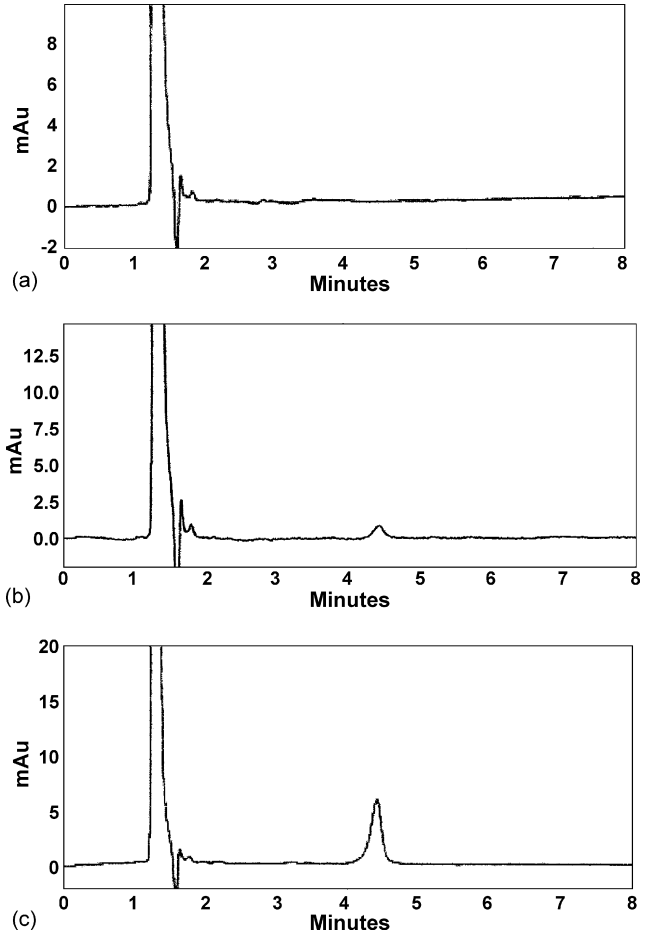

Fig. 4. Representative chromatograms of: (a) blank medium, (b) a 0.16 μg ml^{-1} sample and (c) a 4 μg ml^{-1} sample.

Table 4
Precision and accuracy data for naltrexone

Set number	Nominal concentration (μg ml^{-1})			
	0.45	4.00	8.00	18.00
1	0.45	3.94	7.93	17.94
	0.46	4.06	7.87	17.70
	0.44	3.93	7.78	17.65
Mean	0.45	3.98	7.86	17.76
S.D.	0.01	0.07	0.08	0.16
%R.S.D.	2.0	1.7	1.0	0.9
%DFN	−0.4	−0.6	−1.8	−1.3
2	0.46	4.07	8.14	18.13
	0.45	4.05	7.86	17.53
	0.44	4.04	8.17	17.61
Mean	0.45	4.05	8.06	17.76
S.D.	0.01	0.02	0.17	0.33
%R.S.D.	1.5	0.4	2.1	1.8
%DFN	0.2	1.3	0.7	−1.3
3	0.44	4.09	7.89	17.81
	0.44	3.92	7.83	18.25
	0.45	3.94	7.95	17.83
Mean	0.44	3.98	7.89	17.96
S.D.	0.00	0.09	0.06	0.25
%R.S.D.	0.5	2.3	0.8	1.4
%DFN	−1.2	−0.5	−1.4	−0.2
Global calculation				
Mean	0.45	4.00	7.93	17.83
S.D.	0.01	0.07	0.13	0.24
%R.S.D.	1.5	1.7	1.7	1.4
%DFN	−0.5	0.1	−0.8	−1.0

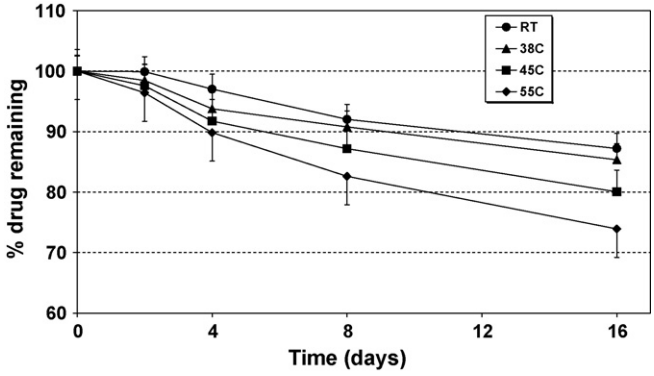

Fig. 5. Loss of naltrexone in drug release medium subjected to different temperature conditions.

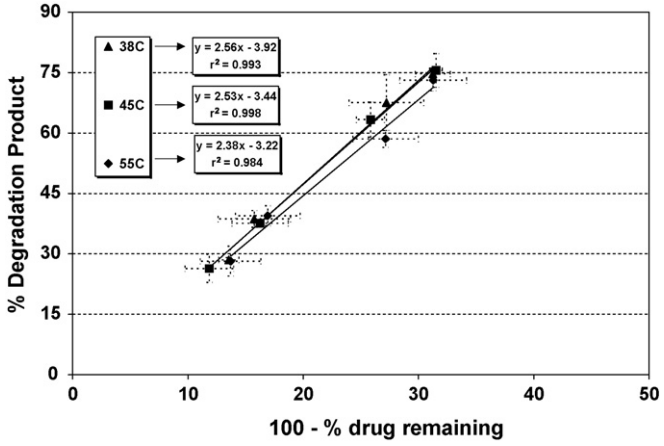

Fig. 6. Product plot of naltrexone at 18 μg ml^{-1} in drug release medium.

3.2.4. Freeze–thaw stability

The freeze–thaw was assessed by a comparison of the peak areas of QC samples injected after one and two cycles of freeze–thaw to the mean peak area of freshly prepared QC samples. A storage duration of 6 days below −20 °C was allowed between each cycle. The mean values up to two freeze–thaw cycles ranged from 97.8 to 105.9% of the initial QC concentration at 0 h (R.S.D. = 0.5–2.3%).

The results also ensured that the study samples could be collected for at least 12 days prior to analysis.

3.2.5. Drug stability

3.2.5.1. Determination of time interval for medium replacement. The stability of naltrexone was tested at 0.4, 4, 8 and 20 μg ml^{-1}. Fig. 5 represents the loss of drug in solution as a function of temperature and time. With an increase in temperature, the degradation of naltrexone increased. The degradation was observed to follow an Arrhenius relationship [$\ln(k) = -2852.4(1/T) + 4.76$; $r^2 = 0.94$], where k is a reaction rate coefficient calculated from the time taken for the drug concentration to reach 90% ($t_{90\%}$) of its initial value and T represents absolute temperature [36]. The activation energy (E_a) calculated from the slope was 5.65 kcal mol^{-1}. This E_a value of less than 10 kcal mol^{-1}, implies the possible involvement of oxidation or photolysis as a potential mechanism for degradation [37,38]. Furthermore, for evaluation of the Arrhenius relationship in making predictions, a technique based on product plots was investigated. As shown in Fig. 6, for naltrexone at 18 μg ml^{-1} level, the percentage appearance of the degradation product plotted against the extent of substrate degradation resulted in almost superimposable lines. Although the plot represents a single concentration level, it indicates that minimal prediction errors are likely from results generated by the Arrhenius equation.

Also, the $t_{90\%}$ values of 10.0, 7.1 and 5.1 days, respectively, at 38, 45 and 55 °C were recorded. Based upon these values, more conservative time intervals of 7, 4 and 3 days, respectively, were fixed for media replacement during studies at those temperatures.

3.2.5.2. Qualitative mass spectral information. Drug degradation was also indicated by the appearance of an additional peak at 3.5 min, found only in the chromatograms of samples following forced degradation (Fig. 3). This peak increased in area as a function of temperature and time. A diode-array spectral scan revealed an absorption wavelength maximum (λ_{max}) of 209 nm for the peak. Another peak was observed at 11 min having a λ_{max} of 225 nm.

The mass spectra yielded peaks with m/z of 358, 342 and 362 at retention times of 8.4, 11.3 and 17.1 min, respectively. The response was too low to allow an MS/MS investigation. However, an m/z ratio of 358 can be attributable to 10-hydroxy naltrexone which, based on a relative retention time of 0.74 is comparable to the value of 0.7 specified in the USP Monograph [22]. The m/z of 362 is attributable to Primocin, as (crosschecked with its solution in water). Other m/z ratios of 239, 261 and 278 were observed and found to correspond to HEPES and its sodium and potassium adducts, respectively. Insignificant intensities of m/z 364 and 380 were also observed which corresponded to sodium and potassium adducts of naltrexone.

3.2.5.3. Quantification of degradation product. For quantification of the degradation product in the absence of a reference standard, a method based on absorptivity changes was employed. A degraded high quality control sample was diluted two- and four-fold with drug release medium, and injected into the HPLC. The resulting areas of naltrexone and its degradant were plotted against drug concentration and the slopes were compared. The ratio of slopes was 0.954, which was then used as a correction factor so that naltrexone could also be employed for quantification of the degradant. The concentration of degradation product increased with an increase in temperature (Fig. 7) and for the 18 μg ml^{-1} sample, a maximum degradation of 1.67 μg ml^{-1} was measured at 16 days of forced degradation at 55 °C. This accounted, in molar terms, for about 40% of the total degradation of the drug at that temperature, indicating the possible existence of multiple pathways for drug degradation that had not been detected at the low concentration of drug employed.

The purpose of development of the HPLC method however, was to provide a reliable and selective quantification of naltrexone in the presence of medium components, and to determine the time interval prior to media replacement during release testing.

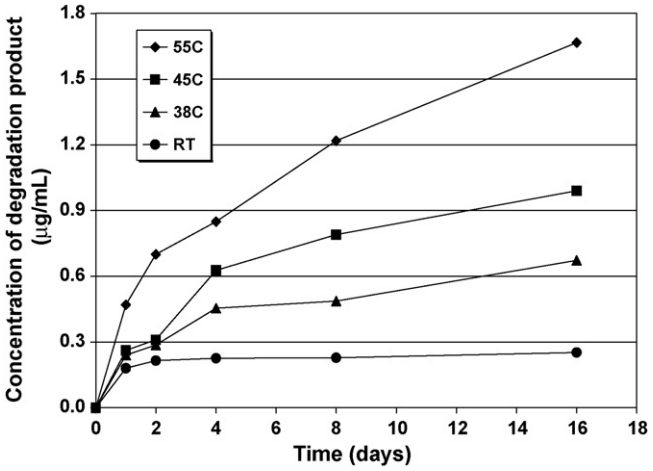

Fig. 7. Quantification of the degradation product formed in a 18 μg ml^{-1} solution of naltrexone in drug release medium when subjected to different temperatures.

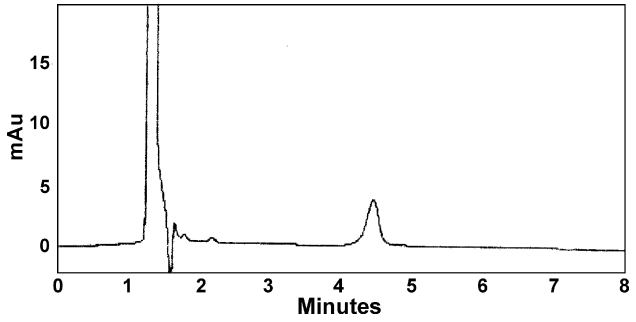

Fig. 8. Chromatogram of day 2 sample of a real-time in vitro release study.

Therefore, further degradation studies to establish mass balance were not conducted.

3.3. Application to samples of an in vitro release study

The modified Hanks' balanced salts solution has been applied for investigation of real-time in vitro naltrexone released from a biodegradable implant supplied by Durect Corporation, CA, USA. The validated HPLC method was used for quantification of these samples. A chromatogram of the day 2 sample is shown in Fig. 8. Approximately 1500 samples have been analyzed thus far using the HPLC method proving its robustness. No chromatographic interference was observed from any degradation products. Also, the peak purity for naltrexone was 99.9%, as estimated using diode-array detection. A description of the 'biorelevant' in vitro drug release study along with pertinent pharmacokinetic data interpretations are addressed in a separate publication.

4. Conclusion

The modified Hanks' balanced salts solution has been shown to be a potential medium for 'biorelevant' in vitro release testing of a naltrexone implant. The physicochemical parameters evaluated for its characterization were within acceptable limits. The HPLC method when applied to real-time samples has generated expected data. This paper potentially serves as a guide for drug development scientists seeking media optimization for 'biorelevant' implant drug release tests, with modification, required on a case-by-case basis. Application of this medium for further studies involving other types of implants may be carried out to extend this approach. Other factors including physicochemical properties of the drug, chromatography, etc. would influence media selection and optimization since the drug has to be sufficiently stable in the medium until analysis.

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