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Optical determination of lithium therapeutic levels in micro-volumes of interstitial fluid

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Abstract

Background: Long-term management of bipolar disorder (BD), characterized by mood fluctuating between episodes of mania and depression, involves the regular taking of lithium preparations as the most reliable mood stabilizer for bipolar patients. However, despite its effectiveness in preventing and reducing mood swings and suicidality, lithium has a very narrow therapeutic index and it is crucial to carefully monitor lithium plasma levels as concentrations >1.2 mmol/L are potentially toxic and can be fatal. Current methods of lithium therapeutic monitoring involve frequent blood tests, which have several drawbacks related to the invasiveness of the technique, comfort, cost and reliability. Dermal interstitial fluid (ISF) is an accessible and information-rich biofluid, and correlations have been found between blood and ISF levels of lithium medication.

Methods: In the current study, we sought to investigate the optical determination of lithium therapeutic concentrations in samples of ISF extracted from porcine skin utilizing a microneedle-based approach. Monitoring of lithium levels in porcine ISF was achieved by employing a spectrophotometric method based on the reaction between the chromogenic agent Quinizarin and lithium.

Results: The resulting spectra show spectral variations which relate to lithium concentrations of lithium in samples of porcine ISF with a coefficient of determination (R^2) of 0.9. This study has demonstrated successfully that therapeutic levels of lithium in micro-volumes of porcine ISF can be measured with a high level of accuracy utilizing spectroscopic techniques.

Conclusions: The results support the future development of a miniaturized and minimally-invasive device for lithium monitoring in bipolar patients.

KEYWORDS
bipolar disorder, interstitial fluid, lithium drug monitoring, personal monitoring, spectrophotometry

1 | INTRODUCTION

Bipolar disorder (BD) is a chronic and recurrent psychiatric illness characterized by mood oscillations, with debilitating episodes of mania and depression intervened with euthymic periods.[1] While BD is classified based on the presence of depression along with manic or hypomanic episodes, there is a concomitant increase in depressive burden with the increase in the severity of mania.[2] Therefore, BD inflicts substantial burdens upon affected individuals due to lost work productivity, increased rates of disability and generally reduced quality of life, therefore more research is essential for alleviating the burden of this debilitating illness.[3] Lithium continues to be prescribed as a first-line mood stabilizer and is the treatment of choice for acute manic, mixed and depressive episodes of BD, in addition to its long-term prophylaxis. However, the use of lithium must be controlled within a narrow therapeutic window of 0.4–1.2 mmol/L in serum blood, as lithium levels above 1.2 mmol/L are potentially toxic and can be fatal.[4] Several methods have been investigated to predict lithium doses required to attain therapeutic levels,[5] including Monte Carlo simulation of lithium concentrations under different missed dose scenarios in patients with normal renal function and renal impairment to achieve suitable lithium replacement dosing schemes.[6] Nevertheless, regular monitoring of blood lithium concentrations is essential to ensure patients are receiving the optimum dose. The current techniques of lithium monitoring including flame emission photometry (FEP) and atomic absorption spectroscopy (AAS) are cumbersome and require frequent blood tests with the consequent discomfort which results in patients evading treatment.[7] Therefore, there is a crucial need to have rapid and simpler methods to determine lithium levels in order to achieve improved rates of lithium treatment monitoring and reduce the chances of adverse events. Most of the previous work on developing a personal lithium monitoring system employs electrochemical methodologies such as ion-selective electrode (ISE), and electrophoresis which have issues related to interference with other ions present in the sample such as sodium, drifting of electrode-electrolyte interface impendence and need for sample filtration.[8–10] Furthermore, the therapeutic monitoring of lithium by these methods requires repetitive withdrawal of blood samples using hypodermic needles which makes sampling invasive and often painful. Efforts have been made to develop minimally invasive point-of-care lithium monitoring devices utilizing matrices such as saliva and sweat.[11,12] However, the results obtained from monitoring the lithium levels in such bodily fluids are not yet reliable, as correlations with the serum levels need to be established. Other limitations of these biological matrices for therapeutic drug monitoring (TDM) include drug instability and the potential presence of contaminants.

Dermal interstitial fluid (ISF), the fluid bathing the viable tissue of the skin, is an accessible and reproducible matrix that has been proposed as a feasible alternative to blood for TDM, as it has a composition similar to plasma[13] and sampling ISF is potentially minimally-invasive and painless.[14,15] Therefore, ISF holds promise as an information-rich and accessible matrix for minimally-invasive measurement of lithium since the concentration of lithium in ISF is suggested to be correlated with venous blood.[13] Leboulanger et al.[13] have demonstrated rapid and precise detection of pharmacokinetic changes in the drug's subdermal concentration and its strong correlation with the corresponding blood level utilizing reverse transdermal iontophoresis which allows quantitative determination of lithium subdermal level without blood measurements. The reverse iontophoretic extraction fluxes of lithium were measured in four successive 30-min periods which showed the strong correlation between systemic lithium concentrations and reverse iontophoretic extraction rates across the skin measured at approximately 90 min after the initiation of the current passage.[13] Therefore, several studies have combined different ISF extraction and lithium monitoring techniques to achieve the determination of lithium levels in ISF. Namely, the combination of potentiometric sensors with reverse iontophoresis (RI), used for extraction of lithium across the skin, has been investigated to achieve the monitoring of lithium in ISF.[13,16] However, these methodologies have several issues including the potential toxicity of sensors, the need for complicated technology, prolonged preparation time and lack of sensitivity. Moreover, minimally invasive collection of ISF remains a challenge as only low volumes of ISF is found in the epidermis and dermis, and the flow of ISF is limited. Recent studies have utilized microneedles, sub-millimetre needle-like structures, which can rupture the first and toughest layer of the skin, the stratum corneum, to access and extract ISF.[17,18] Miller et al.[19] developed a microneedle-based approach for dermal ISF extraction in which the insertion of the microneedle array upon the skin surface induced the ISF flow by creating a local pressure. Similarly, in the current study, we have utilized a facile minimally-invasive microneedle-based approach for dermal ISF extraction from porcine skin. The ISF extraction tool comprises an ultra-fine microneedle connected to a microcapillary tube as well as a syringe to facilitate the flow of ISF based on capillary action upon insertion on porcine skin. So far, our team has successfully developed an optical method that provides highly specific and accurate detection of lithium levels within the narrow therapeutic range in blood. The present work is aimed at using a similar methodology to achieve determinations of lithium concentration in porcine ISF, as porcine stratum corneum is most similar to human stratum corneum and is widely used to study transdermal delivery and skin physiology.[20] Therefore, following microneedle-assisted extraction of ISF from porcine skin, the colourimetric determination of lithium levels in porcine ISF was achieved based on the reaction between chromogenic agent Quinizarin and Li^+ ion which can be detected using optical spectroscopy in the visible region (400 nm–800 nm). Altogether, herein we report spectrophotometric measurements of therapeutic levels of lithium in ISF with an enhanced degree of sensitivity, which serves as the foundation for the development of a miniaturized and minimally-invasive device for continuous lithium drug monitoring.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

1,4-Dihydroxyanthraquinone 96% (Quinizarin), methyl sulfoxide 99.9% ($(CH_3)_2SO$), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) and lithium carbonate 99.9% (Li_2CO_3) were obtained from Fisher Scientific. All solutions were prepared using deionized water (The Deionized Water Company). All analytical grade reagents have been used in this study.

2.2 | Microneedle-based ISF extraction tool fabrication

The microneedle-based ISF extraction tool was designed as a three-component system comprising a single BD 32G 4mm ultra-fine pen needle, a glass capillary attached to the backing of the microneedle and a 10 ml syringe connected to the capillary tube (Figure 1A). Stainless steel BD Ultra-Fine Pen needles (4 mm × 32 G) were obtained from Becton Dickinson. Hirschmann 1–5 µl calibrated pipet capillary tube purchased from Sigma Aldrich was attached to the pen needle. A clear silicone pipe tubing (0.80 mm × 16 mm × 10 mm) was added to the tip of the microcapillary tube prior to its attachment to the pen needle to cover any gaps between the microneedle and the capillary tube and seal the attachment. Thereafter, a 10 ml BD plastic syringe obtained from Fisher Scientific was attached to the other end of the capillary tube which was similarly sealed with a clear silicone pipe tube (Figure 1A). It is important to note that the combination of the microneedle and the glass capillary without the syringe was not sufficient to allow the flow of the fluid based on capillary action. However, attaching a syringe to the other end of the capillary tube and having the syringe plunger pulled, created a negative pressure which allowed extraction of the porcine ISF into the microneedle-capillary sets.

2.3 | Microneedle-assisted extraction of ISF from porcine skin ex vivo

Porcine abdominal skin has been suggested as a suitable model for human skin since it is thinner and less hairy than skin from the dorsal site and allows the microneedles to penetrate into the epidermal region.[20] Therefore, freshly excised porcine abdominal skin

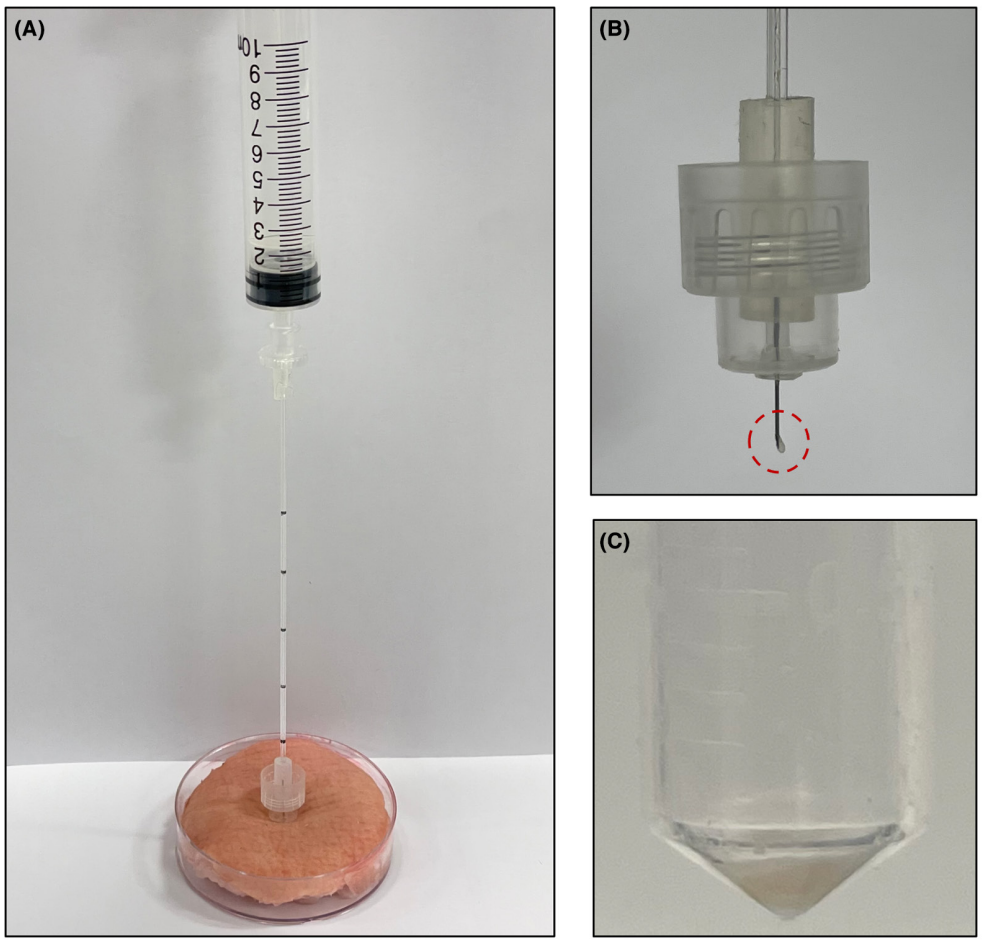

FIGURE 1 (A) Microneedle-based ISF extraction tool. (B) Extracted porcine ISF in the microneedle/capillary glass set before collection into the microcentrifuge for analysis. (C) Sampled porcine ISF (~30 µl).

was obtained from the local slaughterhouse. The skin was cut using a scalpel into circular samples (50 mm diameter) of 3–5 mm thickness. The skin samples were placed in 55 mm Petri dishes (Fisher Scientific) and then kept inside an environmental chamber (Model: KMF 115, Binder GmbH) at 90% relative humidity (RH) and 25°C for 24 h to ensure that maximum hydration levels are reached. Following porcine skin sample preparation, the microneedle-based ISF extraction tool was gently pressed into the skin. The microneedle-based ISF extraction tool was repeatedly applied to the skin surface, thereby inducing the flow of ISF through the micropores into the microneedle-capillary sets following skin puncture. In a typical experiment, moving the ISF extraction tool multiple times across the skin sample allowed for the collection of approximately 26–30 µl of ISF in 1–2 h periods. We have generally observed that while very low volumes of ISF are produced during the first microneedle insertions, the flow of ISF improves as the number of insertions increases. The process of ISF extraction hence affects the rate at which ISF flows potentially by increasing skin hydration and opening the skin micropores after several skin punctures. Thereafter, the ISF was recovered from the microneedle-capillary sets into a microcentrifuge tube (Figure 1C). The collected ISF into the microcentrifuge was topped up with deionized water to the final volume of 100 µl and then centrifuged at 1600 rpm for 1 min. Adding dH_2O to the extracted ISF up to a set final volume allowed us to calculate the amount of sampled ISF.

2.4 | Sample preparation for measurement of lithium levels in porcine ISF ex vivo

A stock solution of 10 mmol/L Lithium was prepared by dissolving 0.0738 g Li_2CO_3 in 100 ml dH_2O. The Li_2CO_3 solution was then further diluted to make two sets of solutions including 10 concentrations each and ranging from 0.2 to 2 mmol/L in the order of 0.2 mmol/L, and 0.4–4 mmol/L in the order of 0.4 mmol/L. Thereafter, samples of porcine ISF extracted earlier were spiked with lithium by mixing 2.5 µl from each concentration of Li_2CO_3 with 2.5 µl of porcine ISF. Therefore, 20 samples of 5 µl spiked porcine ISF with lithium concentrations ranging between 0.1 and 1 mmol/L in the order of 0.1, and 0.2–2 mmol/L in the order of 0.2 were achieved. The different concentrations of Li_2CO_3 in the prepared solutions and in porcine ISF samples are listed in (Table 1).

2.5 | Reference flame photometry measurements on lithium-spiked ISF samples

Samples of lithium solutions, which were prepared to give Li^+ concentrations of 0.2 to 4.0 mmol/L Li^+ in porcine ISF, were first verified through FEP measurements (Table 1). A flame photometer (M410 Sherwood Scientific Ltd) was used to measure the concentration of dissolved lithium in the porcine ISF samples. To perform the reference measurements, the instrument was first left to warm up with the flame alight, and deionized water was aspirated for approximately 30 min. A standard solution provided by the manufacturer (containing 1.5 mmol/L of Li^+) was then aspirated for 30 s and the instrument reading was set to 1.5 mmol/L. Measurements of known lithium concentrations from the prepared solutions were recorded (Table 1). It should be noted that since there are two lithium ions in one molecule of Li_2CO_3, FEP Li^+ measurements yielded concentrations which were approximately twice the prepared Li_2CO_3 concentrations; nonetheless, giving us the lithium levels within the therapeutic range. Since FEP is considered the standard in measuring lithium, the remainder of the analysis was performed using values extrapolated from the flame photometer measurements.

TABLE 1 FEP measurements of lithium concentrations in porcine ISF.

Li_2CO_3 solutions (mmol/L)	Li_2CO_3 concentration (mmol/L) in porcine ISF	FEP Li^+ measurements (mmol/L)
0.2	0.1	0.25
0.4	0.2	0.50
0.6	0.3	0.75
0.8	0.4	1.00
1.0	0.5	1.25
1.2	0.6	1.50
1.4	0.7	1.75
1.6	0.8	1.95
1.8	0.9	2.15
1.0	1.0	2.35
0.4	0.2	0.40
0.8	0.4	0.80
1.2	0.6	1.15
1.6	0.8	1.55
2.0	1.0	1.90
2.4	1.2	2.25
2.8	1.4	2.57
3.2	1.6	2.87
2.6	1.8	3.20
4.0	2.0	3.50

2.6 | ISF collection from porcine skin samples rehydrated with lithium in dH_2O

Since the amount and speed of ISF collection could depend on the skin porcine skin sample, we also performed a brief skin pretreatment by hydrating porcine skin samples with solutions of lithium in dH_2O. This allowed us to both optimize the ISF collection by artificially hydrating the porcine skin samples and also investigate whether lithium can be detected in the collected ISF samples using our optical method which is further elaborated in section 3.3. In order to achieve this, six circular samples (50 mm diameter, 3–5 mm

TABLE 2 Predicted concentrations of Li^+ in ISF extracted from porcine skin samples artificially rehydrated with Li_2CO_3 in dH_2O.

Samples of ISF with unknown Li^+ Concentration	Li_2CO_3 concentration (mmol/L) used for porcine skin hydration	Predicted Li^+ concentrations (mmol/L)
Sample 1	10	0.19
Sample 2	30	0.27
Sample 3	50	0.47
Sample 4	70	1.12
Sample 5	90	0.30
Sample 6	100	0.44

thickness) of porcine abdominal skin were placed in 55-mm Petri dishes. To rehydrate the porcine skin tissue, the samples were covered with 15 ml of lithium in dH_2O solutions with concentrations of Li_2CO_3 ranging from 10 to 100 mmol/L for each sample (Table 2). Thereafter, Petri dishes containing porcine skin samples covered with different concentrations of lithium in dH_2O were kept inside the environmental chamber at 60% relative humidity (RH) and 32° C overnight (~16 h). Optimum humidity and temperature were determined by trial and error to ensure maximum rehydration and freshness of the samples obtained. Following the rehydration of skin samples with lithium solutions, the ISF was extracted using the microneedle-based ISF extraction tool. We observed that artificially hydrating the porcine skin samples prior to ISF extraction, facilitated the ISF flow possibly by enlarging the micropores, thereby allowing easier access to ISF. The extracted ISF samples from the pre-treated porcine skin samples were tested right after extraction to determine the presence of lithium in the ISF using the mentioned spectrophotometric method.

2.7 | Optical measurements of lithium in porcine ISF

To assess the possibility of detecting low concentrations of lithium within the micro volumes of the extracted ISF using optical spectroscopy, 2.5 µl of the sampled ISF was spiked with 2.5 µl of different concentrations of lithium to obtain 20 samples with different concentrations within the therapeutic range (Table 1). However, lithium does not have any optical signature in the spectral region since, like alkali metals, it has relatively poor chemical coordination. Nevertheless, the high charge density of lithium provides great affinity to ligands with donor oxygen, and several chromogenic organic reagents such as 1,4- dihydroxyanthraquinone (Quinizarin) can be used as complexing agents of Li^+. In the suitable medium, the reaction of Li^+ ion with Quinizarin yields a bluish-violet colour which can be used for colourimetric determination of lithium levels in the visible region. Therefore, a spectrophotometric method, based on the reaction between Li^+ and Quinizarin, was used to achieve optical detection of lithium levels in both samples of ISF spiked with different known concentrations of lithium and ISF samples extracted from porcine skin rehydrated with lithium solutions. 0.1 M of NaOH, 0.25 M of Na2CO3, 99.9% $(CH_3)_2SO$ and 1 mM of Quinizarin in $(CH_3)_2SO$ were used for optical analysis of lithium. To achieve optical determination of lithium levels in porcine ISF, an amount of 5 µl from porcine ISF samples containing different concentrations of lithium was pipetted into a test tube. The experiment was performed in duplicates for each concentration of lithium dissolved in ISF. Additionally, a sample of porcine ISF without lithium was included as the control. Prepared samples of ISF were then mixed with 10 µl of 0.1 M NaOH, 1 µl of 0.25 M Na_2CO_3, 4 µl of dH_2O, 215 µl of 90% $(CH_3)_2SO$ and 5 µl of 1 mM of Quinizarin in $(CH_3)_2SO$. All samples were kept in a thermostatic bath (Grant Instruments TM TC120 Series Heated Circulating Bath) at 25° for 30 mins prior to testing.

The spectra of prepared samples were acquired by a dual beam spectrophotometer (Model: Lambda 1050, PerkinElmer Corp) and using quartz cuvettes of 1 mm path length from Hellma Materials GmbH. The instrument was set up to acquire three spectra from each sample in the spectral region between 400 and 800 nm and at increments of 1 nm. A halogen–tungsten lamp was used as a light source and indium gallium arsenide (InGaAs) and polycrystalline lead sulfide (PbS) detectors were used in the detector system. While the response time for both detectors was set at 0.2 s, the gain setting for PMT was set to "Auto" and a gain of 1 was set for the InGaAs detector. The reference and sample attenuators were both set to 100% and the reference cuvette was kept blank at all times for all the samples. To remove any effect of the ambient environment on the spectra, baseline correction of 100% transmittance/0% absorption was introduced in the spectrophotometer.

2.8 | Data analysis and statistics

Three different spectral datasets including two datasets containing samples with known concentrations of lithium (Table 1), and a dataset including six samples with unknown concentrations of lithium in ISF from porcine skin samples rehydrated with different concentrations of lithium in dH_2O (Table 2), were included in this study. Moreover, 0 mmol/L Li^+ was used as the control. Spectra collection and visualization were carried out using UVWin Lab for LAMBDA 1050 (Perkin Elmer) and Spectragryph optical spectroscopy software. Further preprocessing and analysis, including regression analyses, of the spectra, were performed in MATLAB R2020b, MathWorksTM and the PLS-toolbox (Eigenvector Research Inc) Matlab® add-on. The three spectra acquired from each sample were averaged, and pre-processing of all three sets of spectra was performed both separately and in combination. Initially, for each dataset, a spectral difference of the blank (0 mmol/L concentration of Li^+) from all other spectra was performed. This step was followed by baseline correction and Savitsky-Golay (SG) algorithms for both smoothing and second derivatives. These pre-treated spectra were then analysed using linear regression on observed peaks to demonstrate varying concentrations of lithium in each dataset. Thereafter, the two datasets containing 20 known concentrations of lithium were

combined and linear regression was performed on observed peaks relevant to lithium concentrations. Thereafter, all three datasets containing known and unknown concentrations of lithium were combined. A SG derivation was performed to further intensify and emphasize the spectral absorption features and to reduce noise. The values of the polynomial order, derivative and filter width of the SG filter were maintained at 2 and 19, respectively. The spectrum of the sample with 0 mmol/L Li^+ was then subtracted from the rest of the spectra to acquire the spectral difference. This was followed by a Multiple Linear Regression (MLR) analysis to build a model and predict the concentrations of unknown samples of lithium. The coefficient of determination (R^2) values were used to determine the accuracy of the regression models.

3 | RESULTS

3.1 | Therapeutic concentrations of lithium can be detected in micro volumes of spiked samples of porcine ISF using optical spectroscopy

Spectral data of lithium-spiked porcine ISF samples in the presence of Quinizarin, acquired using visible spectroscopy and in the spectral region from 400 to 800 nm, are shown in (Figure 2A,B). For better visualization of concentration variations, the spectra of the two different sets are demonstrated separately. Moreover, to manifest the peaks of porcine ISF samples spiked with therapeutic concentrations of lithium (0.25–3.5 mmol/L), the spectrum representing the sample containing no lithium was subtracted from the others and the difference spectra are illustrated (Figure 2A,B). The observed peaks are assigned to the reaction between the chromogenic agent Quinizarin and the Li^+ present in the sample, with two prominent absorption bands around 550–575 nm and 590–615 nm. These prominent bands represent the absorbed complementary colour to the bluish-violet colour yielded from the reaction between Li^+ and Quinizarin.

From the difference spectra illustrated in (Figure 2A,B), it can be determined that the variations in absorption values seem to be proportional to the concentrations of lithium. In accordance with Beer–Lambert law, with the increase in concentrations of lithium in the spiked samples of porcine ISF, there is an increase in absorption values in the identified regions. Therefore, the variations in absorbance values gave an understanding of the linear correlations between the observed peaks and concentration changes of lithium. Having the peaks identified in the spectra, linear regression was performed on both peaks using selected wavelengths and for both datasets independently (Figure 2C–F). Optical wavelengths which showed the highest regression coefficients were used for dual-wavelength optical analysis to eliminate the need for broad range spectral data which can complicate the implementation of this method into a simple and personal monitoring device. From the two prominent regions 550–575 nm and 590–615 nm, 560 nm and 605 nm were investigated, respectively. Linear Regression was then performed on the absorption values corresponding to the wavelengths of interest. These wavelengths, as expected, correlated linearly for both of the datasets to the concentration of lithium present in the sample, with a high coefficient of regression values depicted in (Figure 2C–F).

In order to further investigate this correlation, the data from two sets of lithium-spiked samples of porcine ISF were combined. The second derivative spectra of 20 different therapeutic concentrations of lithium in porcine ISF are depicted in (Figure 3A). Since the second derivative gives negative peaks for each crest and trough in the spectrum, the variations in absorption minima between 550–570 nm and 595–615 nm regions correlate with the concentrations of lithium in porcine ISF. Linear regression analysis was then performed using the same identified wavelengths (560 and 605 nm) to achieve dual wavelengths optical analysis. The regression analyses shown in (Figure 3B,C), illustrate that minima values, and in turn absorbance, varied in correspondence with lithium concentrations with a high correlation of $R^2 = 0.99$. The highest R^2 value is hence achieved from performing linear regression analysis on pre-processed combined datasets including 20 different concentrations of lithium. Altogether, the absorbance values for each concentration of lithium could be seen as varying as an increasing trend in the difference spectra and as a decreasing trend in the second derivative spectra. Moreover, the Coefficient of Determination of 99% achieved from the regression analyses suggests the successful determination of different concentrations of lithium in porcine ISF. These results show that using the wavelengths pertinent to the reaction between Li^+ ion and Quinizarin in the visible spectral region, the concentration of lithium can be predicted with high levels of accuracy.

3.2 | Lithium can be detected in ISF extracted from porcine skin samples following rehydration in lithium solutions

So far, we have demonstrated accurate detection of lithium levels in ISF which was achieved by spiking samples of extracted porcine ISF with therapeutic concentrations of lithium ex vivo. Thereafter, we sought to investigate the feasibility of detecting lithium within the extracted ISF after artificially rehydrating our porcine skin samples with solutions containing varying concentrations of lithium. When artificially rehydrating porcine skin samples with solutions containing different concentrations of lithium, the amount and rate of lithium perfusion into each skin sample are unknown. Therefore, although porcine skin samples were pre-treated with set concentrations of lithium dissolved in dH_2O, the concentrations of lithium in the extracted ISF samples are unknown and need to be determined. Nevertheless, as illustrated in (Figure 4A), lithium could be detected in the six ISF samples extracted following porcine skin pre-treatment. This shows the feasibility of our colourimetric method for the accurate detection of lithium within the ISF matrix. Therefore, the spectral data of six porcine ISF samples with unknown concentrations of lithium were combined with data from the 20 samples containing known concentrations of lithium. Figure 4B, depicts the predicted Li^+ concentrations

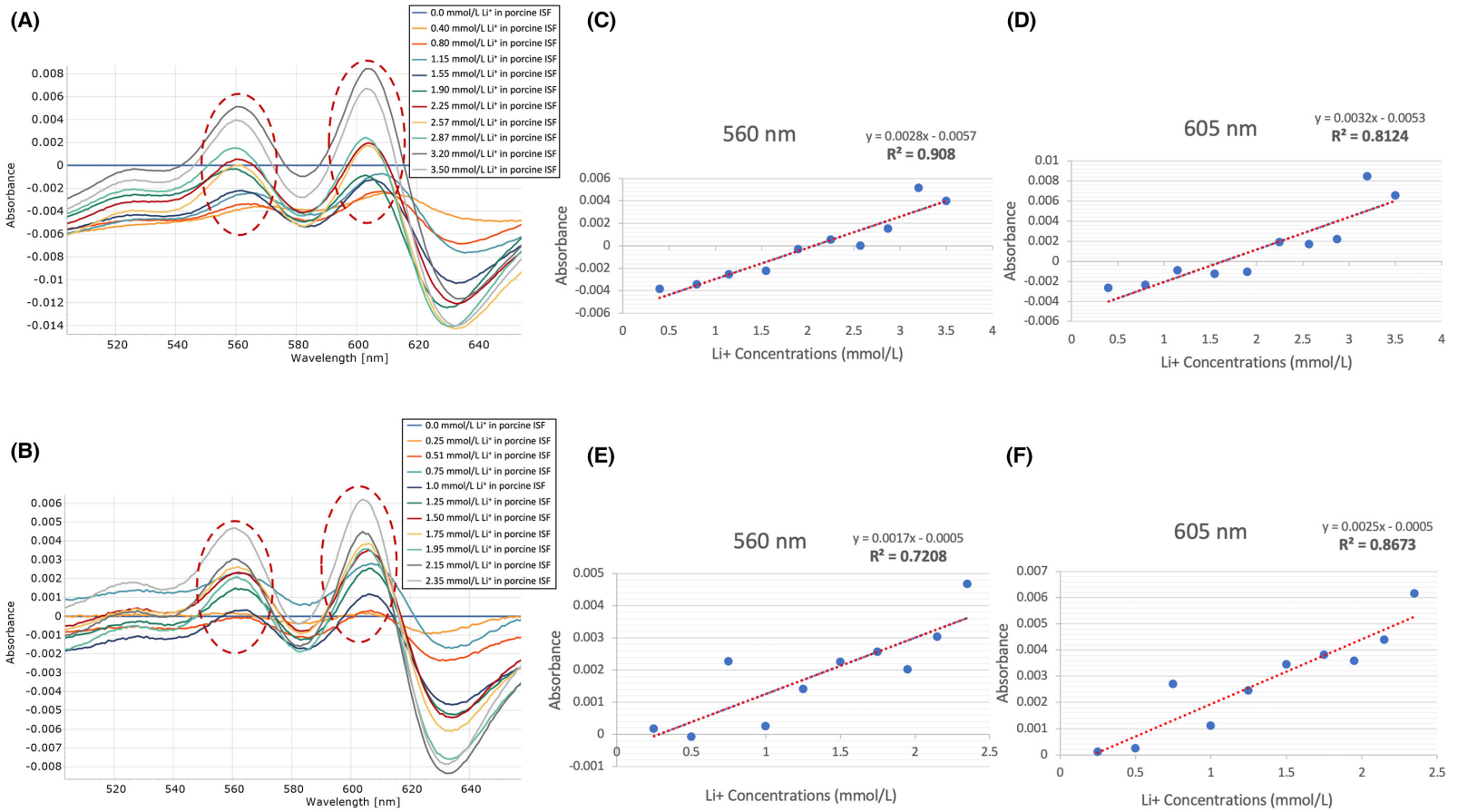

FIGURE 2 Difference spectra of therapeutic concentrations of lithium (data-set one: 0.4–3.5 mmol/L Li+) (A), and (data-set two: 0.25–2.35 mmol/L Li+) (B) in porcine ISF, tested using spectrophotometry based on the reaction of lithium-ion with Quinizarin. (C) Absorption variations of dataset one at 560 nm represent the first peak. (D) Absorption variations of dataset one at 605 nm represent the second peak. (E) Absorption variations of data-set two at 560 nm represent the first peak. (F) Absorption variations of data-set two at 605 nm represent the second peak.

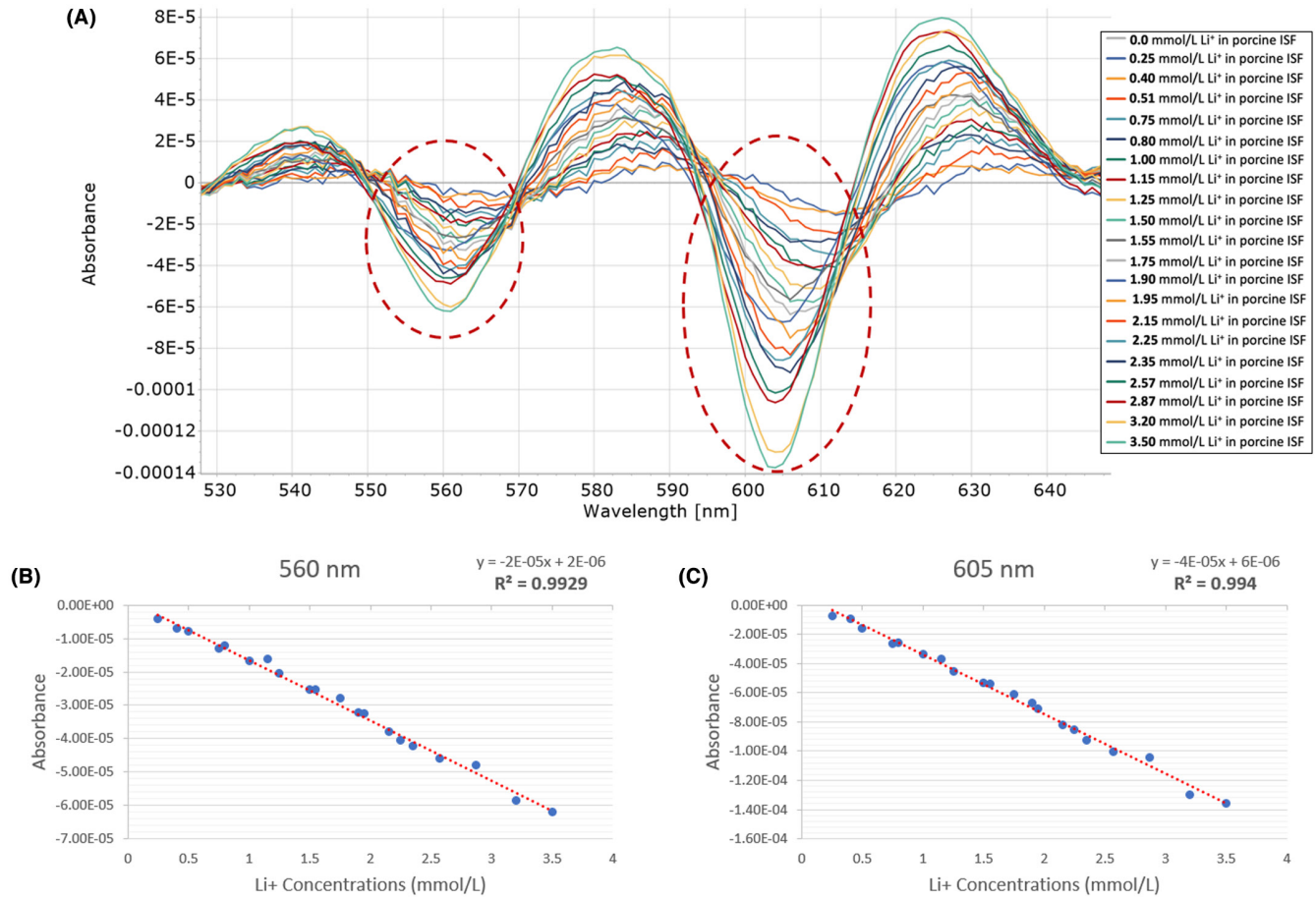

FIGURE 3 (A) Second derivative spectra of therapeutic concentrations of lithium in porcine ISF in the range 530–650 nm after undergoing pre-treatment of baseline corrections, Savistky-Golay filtering and normalization, with prominent bands found at 605 nm and 560 (circled). (B,C) Linear regression analysis of absorption variations of 20 different lithium concentrations at 560 nm (B), representing the first peak; and 605 nm (C), representing the second peak.

in the six ISF samples extracted from pre-treated porcine skin samples. Different concentrations of lithium used for skin pre-treatment for each sample, as well as the predicted lithium in the extracted ISF from each sample, are reported in (Table 2). As demonstrated in (Figure 4B), for skin samples rehydrated with 90 and 100 mmol/L of Li_2CO_3, lower concentrations of Li^+ were detected in the extracted ISF compared to skin samples rehydrated with lower concentrations of Li_2CO_3, this is potentially due to the fact that the lithium has not been perfused into the skin samples at a fixed rate.

3.3 | Identified spectral regions can be used for the predictive modelling of lithium concentrations

As aforementioned, the concentrations of lithium detected in ISF extracted from pre-treated porcine skin samples are unknown. Moreover, due to the low volume of the extracted ISF (5 µl) from each porcine skin sample, the reference flame photometry technique cannot be used to measure the concentration of Li^+ present in the sample. Therefore, to predict the concentrations of lithium in the six porcine ISF samples with unknown Li^+ content, a multiple linear regression (MLR) model was constructed using datasets containing known concentrations of lithium. The full range data of pre-treated spectra between 530 and 650 nm were used for lithium concentration prediction using MLR. To build the MLR model using the 20 known concentrations of Li^+, spectral data were used as calibration data input whilst lithium concentrations obtained through FEP measurements were used as the dependent variable (Figure 5A). Moreover, to investigate the accuracy of the model, venetian blinds cross-validation was applied, which ensured that the performance of the model is optimized. The parameters, coefficient of determination, (R^2), and the root mean squared error of cross-validation (RMSECV) are used as indicators for the regression models and are demonstrated in (Figure 5C) which summarizes the prediction statistics and the performance of the regression analysis. The coefficient of determination of 98% achieved from the regression analysis ensures an accurate determination of lithium levels, and the RMSECV value could be an indication that lithium concentration at low values can be predicted accurately. Therefore, the results of regression yielded a very good predictive performance with

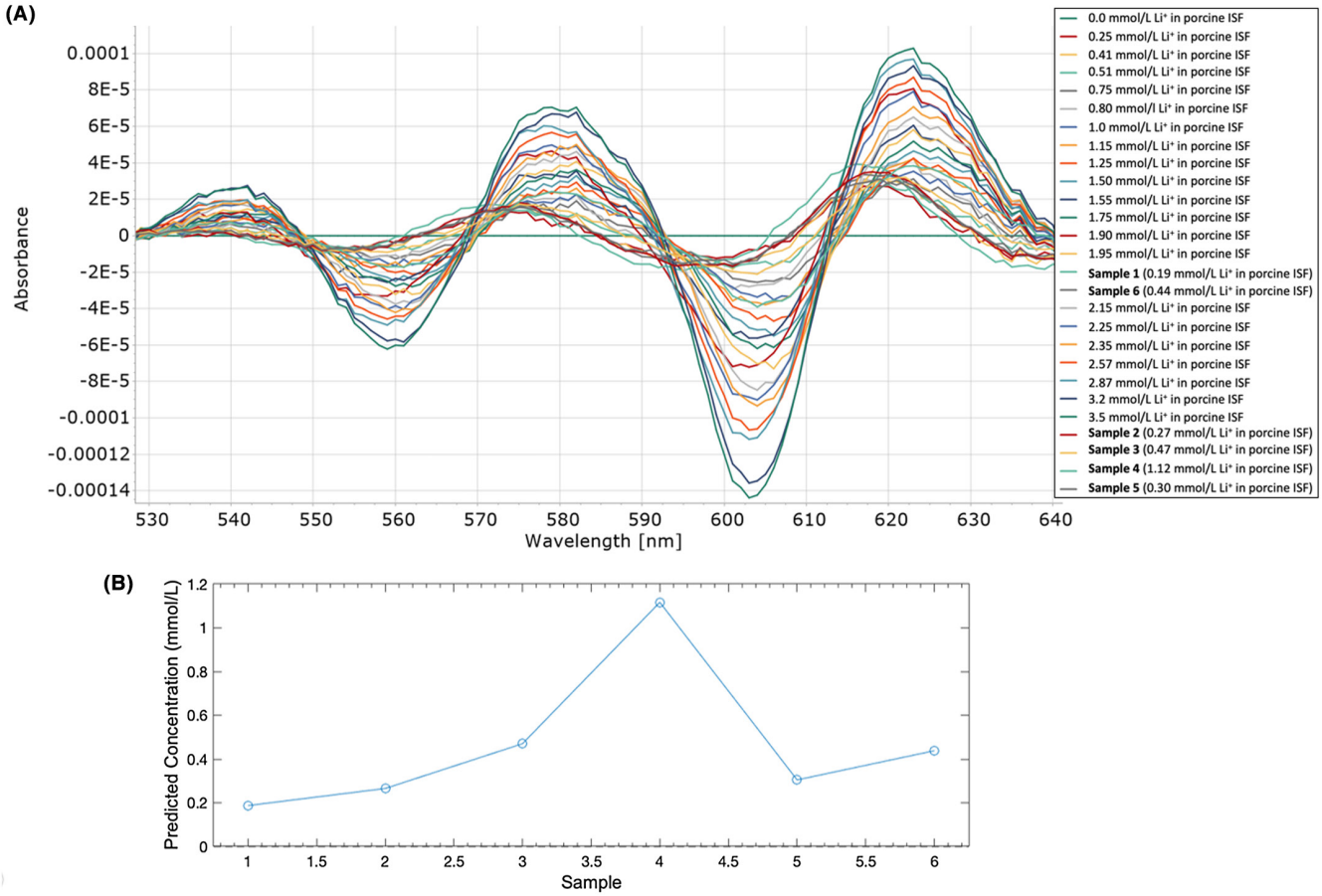

FIGURE 4 (A) Second derivative spectra of known and unknown concentrations of lithium in porcine ISF in the range 530–650 nm after undergoing pre-treatment of baseline corrections, Savistky-Golay filtering and normalization. (B) Predicted Li + concentrations in six samples of porcine ISF containing unknown concentrations of lithium.

R^2 of 0.9873 meaning that changes in lithium concentrations influence the optical absorbance in a linear manner, hence this interrelationship can be modelled using MLR to achieve concentration prediction. The predicted ISF lithium levels resulting from the regression are illustrated in (Figures 5B and 4B). Overall, the results suggest the high performance of the investigated optical method as well as the developed predictive model for the accurate determination of lithium levels in samples of porcine ISF.

4 | DISCUSSION AND CONCLUSIONS

The development of easy-to-use devices for decentralized monitoring of lithium therapeutic levels will be a major advance in the management of the bipolar disorder. However, point-of-care and low-cost monitoring of lithium therapeutic levels in bipolar patients remain a challenge. This study set out with the aim of investigating the feasibility of employing the proposed spectrophotometric methodology, based on the reaction between the chromogenic agent Quinizarin and Li^+, for precise detection of lithium therapeutic levels in samples of ISF. The accessible location of dermal ISF and the correlations found between blood and ISF levels of lithium resulted in our research effort for determining lithium therapeutic concentrations in ISF, which serves as a foundation for the future development of a point-of-care device for therapeutic drug monitoring in bipolar patients.

In the current study, we have developed and combined a microneedle-based ISF extraction technique with a spectrophotometric method, based on the reaction of lithium-ion with the chromogenic agent Quinizarin, to achieve optical detection of lithium levels in micro-volumes of porcine ISF with high levels of accuracy. A major limitation of employing ISF for TDM is extracting a reliable amount of analytes for downstream analysis, as most of the skin's ISF is in the dermis which makes the ISF flow limited as it contains a network of collagen and elastin fibres surrounded by extracellular matrix.[17] Moreover, only extremely low volumes of ISF are found in the epidermis (20 nl/mm^2) and dermis (800 nl/mm^2), which makes the process of extracting this fluid rather difficult.[21] Previously reported ISF extraction methods to include suction blister, dialysis, or sonication, with all having the disadvantage of altering the composition of ISF, due to the local trauma caused by the extraction process.[22–25] A number of studies have also proposed novel ISF extraction methods utilizing microneedle patches.[17,26] Using microneedle (MN) patches collection of >1 μl

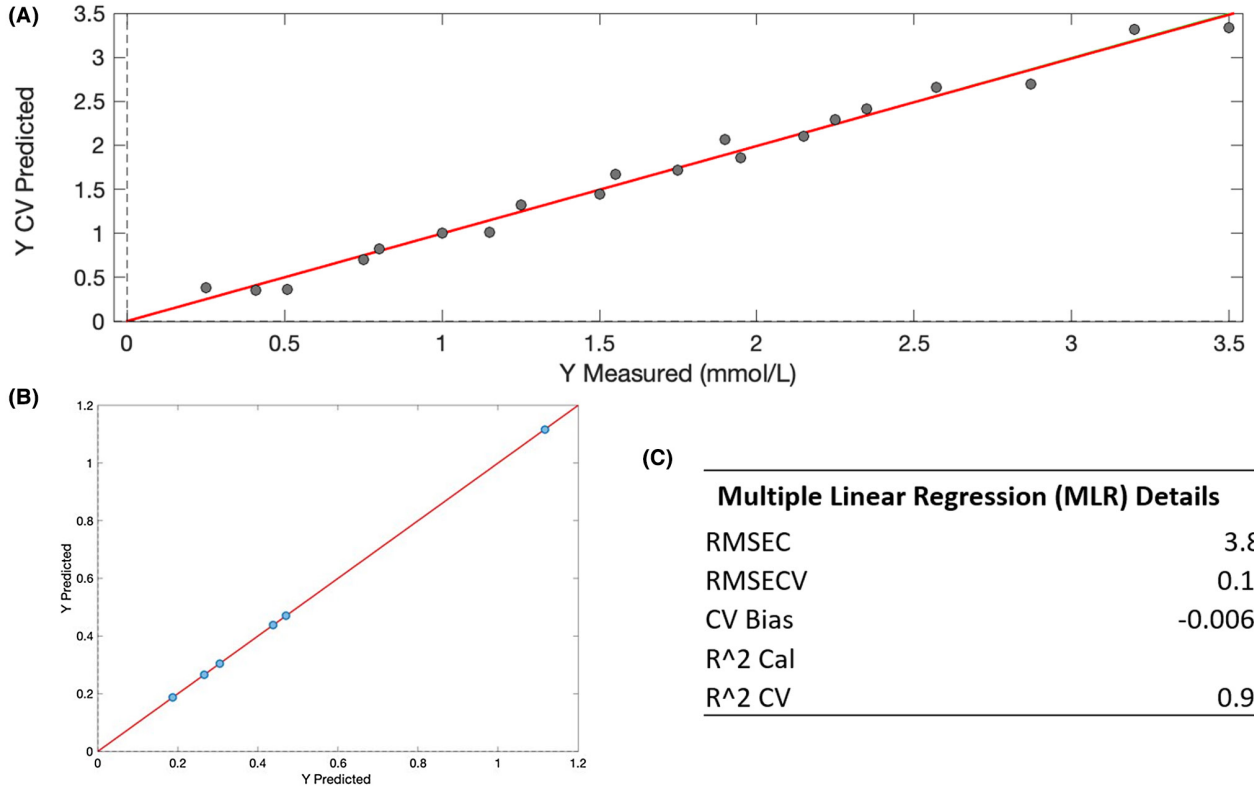

FIGURE 5 Prediction of ISF lithium levels with MLR analysis using feature optical wavelengths (530–650 nm). (A) Measured (FEP) versus predicted concentration of lithium-ion in ISF. (B) Calculated concentrations of unknown lithium levels. (C) Regression details.

of ISF within 20 min in pig cadaver skin and living human subjects,[17] and 2.9 μl within 30 s from the rat skin in vivo[26] have been achieved. Herein, we have utilized a microneedle-based ISF extraction tool fabricated using widely available materials which allowed the extraction of ~26–30 μl of ISF in 1–2 h periods. Overall, utilizing the fabricated ISF sampling tool, we achieved the extraction of relatively high volumes of ISF from porcine skin ex vivo. Additionally, the introduced ISF extraction tool is of great interest for further characterization of skin physiology and pathophysiology and monitoring the dermal interstitium in different skin samples. It should also be noted that more ISF could generally be collected from skin tissue in-vivo than from porcine skin ex-vivo. Lastly, the microneedle's small diameter, used in the fabricated ISF sampling tool, limits pain and penetration force and is coated with a silicone lubricant to minimize tissue trauma upon insertion, hence is suitable for in vivo studies. Therefore, future studies will focus on the optimization of the fabricated ISF extraction tool to ensure minimally invasive ISF extraction in human subjects with a suitable amount of pressure applied.

Following extraction of a sufficient amount of ISF from porcine skin, we sought to achieve optical determination of therapeutic concentrations of lithium in this matrix. Our group has previously reported measurements of lithium levels in blood using optical spectroscopy techniques with a high degree of accuracy and selectivity.[27] The findings observed in this study further support the feasibility of employing the investigated methodology for the accurate detection of lithium levels in micro-volumes of ISF. While extremely low volumes of ISF can be extracted from the skin, the majority of the available methods for lithium therapeutic monitoring require large sample volumes. The optical method investigated in the current study, however, demonstrated accurate detection and quantification of lithium in samples of porcine ISF as small as 5 μl. As elaborated in section 3, results of the linear regression analyses performed using only feature wavelengths of optical measurements and again using the proposed method showed that the absorption values are linearly correlated at a high level of coefficient of determination (R^2) to the concentration of lithium present in the sample. Moreover, using ISF samples with unknown lithium content, the spectrophotometric method provided remarkably good prediction of lithium content with more than 90% accuracy. Detection of unknown concentrations of lithium in porcine ISF was also achieved by artificially rehydrating porcine skin samples with different concentrations of Li_2CO_3 in dH_2O prior to ISF extraction. However, from the predicted concentrations of Li^+ detected in ISF extracted from each sample of porcine skin (Table 2), it can be postulated that lithium has not been perfused into the skin samples at a fixed rate. Therefore, although the method of pre-treating porcine skin samples with solutions containing different concentrations of lithium, facilitated the ISF extraction and allowed the detection of unknown concentrations of lithium within the ISF, future studies should investigate different tissue permeation techniques to ensure lithium is perfused at a fixed rate.

In conclusion, the undertaken studies have demonstrated the feasibility of the proposed spectrophotometric method for

the precise determination of lithium levels in low volumes of ISF using few feature wavelengths only, which serves as a basis for our studies in this matrix in human subjects. Monitoring of analytes of interest in the skin compartment will be the focus of the next generation of devices for personal healthcare monitoring. Extraction and on-device analysis of ISF for TDM using the proposed optical method will enable the development of a miniaturized and minimally invasive device for lithium drug monitoring. Ultimately, continuous monitoring of lithium in the ISF would offer a more informative measure of this metabolite and will allow bipolar patients to monitor their medication which will greatly reduce the risk of adverse effects.

AUTHOR CONTRIBUTIONS

Mahsa Sheikh (M.S.): writing—original draft, visualization, conceptualization, data Curation, investigation, validation, methodology. Mahsa Sheikh (M.S.) and Meha Qassem (M.Q.): conceptualization and methodology. Meha Qassem (M.Q.) and Panayiotis A. Kyriacou (P.A.K.): validation, investigation and supervision.

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DATA AVAILABILITY STATEMENT

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