UNIVERSIDADE FEEVALE

MESTRADO ACADÊMICO EM TOXICOLOGIA E ANÁLISES TOXICOLÓGICAS

EMPREGO DE MANCHAS DE SANGUE SECO EM PAPEL E PLASMA SECO EM PAPEL NO MONITORAMENTO DA TERAPIA COM CARBONATO DE LÍTIO: DESENVOLVIMENTO DE METODOLOGIA ANALÍTICA E APLICAÇÃO CLÍNICA

IURI DIAS MANFRO

Linha de pesquisa: Toxicologia Humana e Análises toxicológicas

Professor (a) orientador (a): Profª. Drª. Marina Venzon Antunes

Novo Hamburgo, julho de 2019

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IURI DIAS MANFRO

Dissertação intitulada EMPREGO DE MANCHAS DE SANGUE SECO EM PAPEL E PLASMA SECO EM PAPEL NO MONITORAMENTO DA TERAPIA COM CARBONATO DE LÍTIO: DESENVOLVIMENTO DE METODOLOGIA ANALÍTICA E APLICAÇÃO CLÍNICA apresentada ao Programa de Pós-Graduação em Toxicologia e Análises Toxicológicas, da Universidade Feevale, como requisito necessário

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RESUMO

O carbonato de lítio (Li) é o estabilizador de humor mais utilizado e efetivo no tratamento do transtorno afetivo bipolar (TAB), possui índice terapêutico estreito e, apesar de eficaz, é frequentemente associado à ocorrência de efeitos adversos. A toxicidade ao Li pode estar relacionada às características farmacocinéticas individuais e às interações medicamentosas, contribuindo para a baixa adesão ao tratamento. Na busca pela eficácia terapêutica é indispensável o monitoramento dos níveis séricos de Li, principalmente na farmacoterapia combinada com outros fármacos e em pacientes com reduzida depuração renal, como idosos. O uso de manchas de sangue seco em papel (DBS) e de manchas de plasma seco em papel (DPS), surgiu como estratégia alternativa de amostragem no monitoramento terapêutico de fármacos (MTF), devido à sua praticidade na coleta e no transporte e pela conservação da estabilidade dos analitos. Entretanto, até o momento não há descrição na literatura do uso de amostras de DBS e DPS no monitoramento da exposição sistêmica ao Li. Em virtude das vantagens apresentadas por estas matrizes, o presente trabalho teve como objetivo desenvolver e validar os métodos de DBS e DPS, empregando Espectrometria de Absorção Atômica com Forno de Grafite (EAAFG) para aplicação clínica no monitoramento da farmacoterapia de pacientes com TAB. O método foi linear para ambas as matrizes apresentando valores de r superiores a 0,99. Os ensaios de precisão tiveram CV% de 3,6 a 7,2% para as amostras de DBS e de 4,6 a 9,3% para as amostras de DPS e exatidão entre 97 a 109% e 98 a 106% para DBS e DPS respectivamente. O limite inferior de quantificação foi de 0,10 mEq/L para ambas as matrizes, com CV% intra-ensaio de 11% e inter-ensaio de 14% e exatidão de 104% para DBS e CV% intra-ensaio de 20% e inter-ensaio de 18% com exatidão de 113% para DPS. Não foram detectados interferentes nas amostras de DBS e DPS isentas de Li. O Li mostrou-se estável durante 20 dias em DBS e DPS nas temperaturas de -20, 25 e 42°C apresentando valores de 87 a 110% e de 86 a 113% respectivamente. Ambos os métodos de amostragem foram desenvolvidos, validados e aplicados na determinação de Li em DBS e DPS obtidas de 43 pacientes, juntamente com a dosagem sérica. As concentrações de Li medidas em DBS e DPS foram correlacionadas com as concentrações séricas, apresentando correlações significativas com valores de r entre 0,734 e 0,866 respectivamente, porém não foi possível predizer as concentrações séricas a partir dos níveis sanguíneos de Li. A partir das dosagens em DBS e das medidas em DPS é possível estimar os níveis eritrocitários de Li. Sendo assim, ambos os métodos de amostragem poderiam ser considerados na busca pela expansão do monitoramento da farmacoterapia com Li.

Palavras-chave: Carbonato de lítio, monitoramento terapêutico de fármacos, validação, manchas de sangue seco em papel, manchas de plasma seco em papel.

ABSTRACT

Lithium carbonate (Li) is the most used mood stabilizer and effective in bipolar disorder treatment. Li have a narrow therapeutic index and though efficient is frequently associated to side effects occurrence. Li toxicity could be related to individual pharmacokinetic characteristics and drug interactions, contributing for low treatment compliance. In therapeutic efficacy search is paramount monitoring of Li serum levels, mainly agreed pharmacotherapy with other drugs and in patients with low renal clearance such as elderly people. Dried Blood Spots (DBS) and Dried Plasma Spots (DPS) use raised such as alternative sampling strategies in therapeutic drug monitoring (TDM), due your collect and transport convenience and stability conservation of analytes. Even though, up to now not either have description of DBS and DPS samples utilization in monitoring of Li systemic exposition. Due to advantages presented for these matrices, the aim of this study was to develop and validate DBS and DPS assays, employing Graphite Furnace Atomic Absorption Spectrometry (GFAAS) for clinical application on the pharmacotherapy monitoring of patients with bipolar disorder. The method was linear from 0.10 to 3.0 mEq/L for both matrices, presenting r values higher than 0.99. Precision assays presented CV% of 3.6-7.2% for DBS samples and 4.6-9.3% for DPS samples and accuracy in the range of 97 to 109% and 98 to 106% for DBS and DPS samples respectively. Lower limit of quantification was 0.10 mEq/L for both matrices with CV% intra-assay of 11% and inter-assay of 14% and accuracy of 104% for DBS and CV% intra-assay of 20% and inter-assay of 18% and accuracy of 113% for DPS. No interfering peaks were detected in blank DBS and DPS samples. Li was stable in DBS and DPS samples up to 20 days at -20°C, 25°C and 42°C, presenting values between 87 to 110% and 86 to 113% for DBS and DPS respectively. Both assays were developed, validated and applied in determination of Li in DBS and DPS obtained from 43 patients, closely with serum measurement. DBS and DPS Li concentrations were correlated with serum Li concentrations, presenting meaningful correlation among these matrices with r values between 0.734 to 0.866 respectively, however was not possible predict serum Li concentrations stem from Li blood measurements. According to results, is possible claim both sampling methods could be considered in search for monitoring expansion of pharmacotherapy with Li.

Keywords: Lithium carbonate, therapeutic drug monitoring, validation, dried blood spots, dried plasma spots.

LISTA DE ABREVIATURAS E SIGLAS

AGNP: Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie

AINES: Antiinflamatórios não-esteroidais

AMP: *Adenosine monophosphate*

Bcl-2: *B cell lymphoma protein-2*

BDNF: *Brain-Derived Neurotrophic Factor*

BPNase: Bifosfato nucleotidase

Ca^{2+}: Cálcio

CLss: Lítio sérico em equilíbrio dinâmico

COX: Ciclooxigenases

D: Dose

DBS: *Dried Blood Spots*

DI: Dose individualizada

DPS: *Dried Plasma Spots*

EAAFG: Espectrometria de Absorção Atômica com Forno de Grafite

FDA: *Food and Drug Administration*

FBPase: Frutose 1,6 bifosfatase

GABA: Ácido gama-aminobutírico

GSK-3: *Glycogen Sinthase Kinase-3*

HCT: Hematócrito

IMPase: Inositol monofosfatase

IECA: Inibidores da Enzima Conversora de Angiotensina

IMAO: Inibidores de Monoaminaoxidase

ISRS: Inibidores Seletivos da Recaptação de Serotonina

IPPase: Inositol polifosfato 1-fosfatase

K^{+}: Potássio

Li: Carbonato de lítio

LiR: *Lithium ratio*

MTF: Monitoramento Terapêutico de Fármacos

Na^{+}: Sódio

SNC: Sistema Nervoso Central

TAB: Transtorno Afetivo Bipolar

SUMÁRIO

1. APRESENTAÇÃO GERAL

O presente trabalho teve como objetivo desenvolver e validar metodologias analíticas para a dosagem de lítio em amostras de DBS e DPS, visando calcular o índice de lítio e as concentrações eritrocitárias a partir das obtidas nessas duas matrizes e avaliar seu desempenho na avaliação da exposição sistêmica ao lítio. O referido trabalho tem início com uma breve revisão bibliográfica sobre a pesquisa realizada, seguida da apresentação de um capítulo composto por um artigo científico encaminhado para publicação como relatado a seguir:

CAPÍTULO 1: Artigo preparado segundo as normas da revista Talanta, intitulado "*Determination of lithium in Dried Blood Spots and Dried Plasma Spots by Graphite Furnace Atomic Absorption Spectrometry: Method development, validation and clinical application"*.

Durante a pesquisa, trabalhos foram apresentados em eventos científicos, a saber:

- *"Development and validation of a method for the determination of lithium in Dried Blood Spots by graphite furnace atomic absorption spectrometry"*. Apresentado na forma de resumo/pôster no II Congresso Latino-americano de Toxicologia Clínica e Laboratorial, realizado na Universidade Federal do Rio Grande do Sul em Porto Alegre/RS entre os dias 03 a 06 de junho de 2018.

- *"Desenvolvimento e validação de um método para a determinação de lítio em plasma seco em papel por espectrometria de absorção atômica com forno de grafite"*. Apresentado na forma de resumo/pôster no XVI Congresso Brasileiro de Biomedicina e IV Congresso Internacional de Biomedicina, realizado na Universidade Nove de Julho em São Paulo/SP entre os dias 05 a 08 de setembro de 2018.

- *"Determinação de lítio em manchas de sangue seco em papel por espectrometria de absorção atômica com forno de grafite".* Apresentado na forma de resumo expandido no Seminário de Pós-Graduação-Inovamundi 2018, realizado na Universidade Feevale em Novo Hamburgo/RS entre os dias 22 a 27 de outubro de 2018.

- *“Avaliação da adesão de pacientes com transtorno bipolar e em tratamento com carbonato de lítio”.* Apresentado na forma de resumo/pôster no I Congresso Latino-americano de Biomedicina e Ciências do Laboratório, XIII Congresso Sul brasileiro de Biomedicina e IV Congresso Catarinense de Biomedicina, realizado no Centro de Eventos Governador Luiz Henrique da Silveira em Florianópolis/SC entre os dias 01 a 04 de maio de 2019.

2. INTRODUÇÃO GERAL

2.1. Carbonato de Lítio (Li)

O carbonato de lítio (Li) foi descoberto em 1949 pelo cientista australiano John Cade e aprovado pela *Food and Drug Administration* (FDA) como o primeiro fármaco para o tratamento do Transtorno Afetivo Bipolar (TAB). O Li está disponível comercialmente como comprimidos de 450 mg de liberação lenta e de 300 mg de liberação rápida. Os regimes com formulações de liberação lenta permitem que a dose seja administrada apenas uma vez ao dia contribuindo para o aumento da adesão ao tratamento. As formulações de liberação rápida, atingem picos plasmáticos imediatos, contribuindo para a ocorrência de efeitos adversos, portanto seu uso deve ser monitorado (HANEMANN, 2010). A dose inicial proposta é de 900 a 1.200 mg/dia, sendo administrada três vezes ao dia por 5 dias, sob monitoramento das concentrações séricas. Para pacientes idosos ou com depuração renal menor que 80 mL/min, a dose recomendada é de 300 mg duas vezes ao dia (ROSA et al., 2006). Para pacientes adultos em tratamento profilático, recomenda-se uma dose de 900 a 1.800 mg/dia e 900 a 2.400 mg/dia para pacientes em tratamento crônico (BRASIL, 2016; DUQUE et al., 2017).

2.2. Farmacocinética e farmacodinâmica do Li

Ao ser ingerido, o Li é absorvido no trato gastrointestinal sem passar por processo de biotransformação, distribuindo-se lentamente (6 a 10 horas) por todos os tecidos e fluidos corporais em velocidades diferentes sem se ligar às proteínas plasmáticas. O pico de concentração máxima do Li ocorre em 15 minutos nos rins mantendo-se estável por até 7 dias, possuindo tempo de meia-vida de 12 horas. É importante ressaltar que o Li atravessa a barreira hematoencefálica lentamente, levando 24 horas para atingir o pico máximo e que seus níveis cerebrais correspondem a 77% dos níveis séricos, lembrando que seu acúmulo pode causar toxicidade. O intervalo terapêutico proposto para o Li no Brasil é de 0,6 a 1,2 mEq/L, sendo estabelecido a partir da dosagem dos níveis séricos. É imprescindível que as concentrações terapêuticas sejam mantidas durante o tratamento com a finalidade de alcançar a eficácia terapêutica, minimizando o risco de episódios maníacos, hipomaníacos e depressivos e a ocorrência de toxicidade (ROSA et al., 2006; BRASIL, 2016).

Severus et al. (2010) realizaram um estudo com pacientes portadores de transtorno bipolar do tipo 1, avaliando o possível efeito de dose nos riscos proporcionais de episódios maníacos, hipomaníacos ou mistos através do modelo de Cox para eventos concorrentes que incorporam os níveis de Li. O grupo de pacientes que apresentou níveis séricos de Li abaixo de 0,6 mEq/L teve um risco aumentado para episódios maníacos, hipomaníacos ou mistos, enquanto que o grupo de pacientes com alto teor de Li não apresentou risco para episódios maníacos, hipomaníacos, mistos ou depressivos. A partir desses resultados os autores concluíram que são necessárias doses acima de 0,6 mEq/L para a prevenção dos episódios maníacos, hipomaníacos ou mistos e de doses superiores a 0,8 mEq/L para a prevenção de episódios depressivos (SEVERUS et al., 2010).

Paton et al. (2010) realizaram um estudo com 2776 pacientes portadores de algum transtorno afetivo no Reino Unido. Desses pacientes, 1919 receberam diagnóstico de TAB, sendo que 90% faziam tratamento com Li há um ano. Os outros 857 pacientes eram portadores de outros transtornos afetivos. Em média 736 mg/dia eram administradas em pacientes com TAB e 598 mg/dia em média em pacientes com outros transtornos afetivos, porém as concentrações séricas dos dois grupos estavam em 0,6 mEq/L, sendo que 80% administravam fármacos psicotrópicos e antidepressivos em associação com Li. 48% dos pacientes com outros transtornos afetivos eram idosos, o que justifica a dose mais baixa e as concentrações séricas similares aos pacientes com TAB, dos quais somente 25% eram idosos. A minoria dos pacientes apresentou elevadas concentrações de Li, acarretando em toxicidade aguda e dano renal permanente a longo prazo, porém relata-se que pacientes com 0,4 mEq/L podem ter recaídas (PATON et al., 2010).

Nierenberg et al. (2013) realizaram um estudo com 283 pacientes, demonstrando a necessidade de doses personalizadas. Os pacientes administraram doses personalizadas (média de 600 mg/dia), sendo monitorados durante 6 meses. Esses pacientes apresentaram concentrações séricas dentro da faixa terapêutica (média de 0,6 mEq/L), cujos resultados foram positivos devido à diminuição do risco de efeitos adversos e ao aumento da eficácia terapêutica, melhorando a adesão ao tratamento (NIERENBERG et al., 2013).

A excreção do Li se dá em 95% pela urina, 4% no suor e 1% nas fezes, mas também pode ser excretado na saliva em concentrações maiores do que as plasmáticas. O Li também pode ser eliminado no leite materno, tornando a amamentação desaconselhável no período do tratamento. Além disso, quase 80% desse íon filtrado é reabsorvido no túbulo proximal, tornando a depuração renal em 20% da creatinina, variando de 15 a 30 ml/min e 10 a 15 ml/min em idosos, devido à diminuição da função renal. Portanto, recomenda-se que a dose

administrada em idosos seja menor. A reabsorção tubular renal do Li impede seu uso concomitante com diuréticos, pois causa diminuição da depuração renal aumentando o risco de toxicidade (HANEMANN, 2010). Durante o tratamento recomenda-se que o paciente beba bastante água, pois pode ocorrer alteração do equilíbrio hídrico-eletrolítico do organismo (ORUCH et al., 2014).

Os efeitos farmacodinâmicos do Li no organismo consistem na inibição de enzimas dependentes de magnésio através da competição pelos sítios de ligação. O Li também se liga aos receptores da proteína-G envolvida na sinalização intracelular e na síntese do AMP cíclico, através da inibição seletiva da enzima adenilil ciclase, desencadeando o efeito antidepressivo (CAN; SCHULZE; GOULD, 2014). O Li inibe as enzimas frutose 1,6 bifosfatase (FBPase), bifosfato nucleotidase (BPNase), inositol monofosfatase (IMPase) e inositol polifosfato 1-fosfatase (IPPase) envolvidas na conversão do mio-inositol em inositol, promovendo o aumento do mio-inositol e a depleção do inositol. O Li também se liga aos receptores da insulina e aos receptores da tirosina-quinase neurotróficos, ativando as proteínas quinases, as quais exercem efeito regulatório sobre a enzima glicogênio sintase quinase-3 (GSK-3) responsável pelo ciclo de vida das células nervosas como desenvolvimento neuronal, estabilidade do citoesqueleto e apoptose. Além disso, as proteínas quinases ativam fatores regulatórios da expressão gênica, aumentando a síntese da proteína Bcl-2 e do fator neurotrófico derivado do cérebro (BDNF) promovendo efeito neurotrófico e neuroprotetor das células nervosas (LIN; HUANG; LIU 2013; CAN; SCHULZE; GOULD, 2014).

2.3. Efeitos adversos do Li

Apesar de ser o tratamento de escolha para o TAB, a terapia com Li está relacionada à ocorrência de efeitos adversos, como náuseas, vômitos, tremores, ganho de peso, apatia, sonolência e rigidez muscular, os quais contribuem para a baixa adesão ao tratamento (ROSA et al., 2006). Estima-se que a taxa de adesão média ao tratamento com Li seja de 22,2%, enquanto que 41,7% dos pacientes não aderem à prescrição médica por comportamento intencional e 36,1% sejam não-aderentes por comportamento não intencional (SOUZA et al., 2013). Esse número não está relacionado somente à ocorrência de efeitos adversos, mas também à falta de conhecimento da doença, à suspensão temporária de doses ou atrasos nos horários de tomada da medicação, ao regime complexo de tratamento e ao uso associado com outros fármacos (ROSA et al., 2006).

Em virtude das características farmacocinéticas individuais, recomenda-se que o paciente seja submetido a uma avaliação médica para evitar contraindicações e a ocorrência de possíveis episódios agudos como: náuseas, vômitos e dores de cabeça. Nesta avaliação devem ser avaliadas características como: idade, peso, altura e pressão arterial, além da realização de exames bioquímicos, hematológicos e de função tireoidiana. Caso ocorram alterações nos exames, o paciente deve ser encaminhado a uma avaliação clínica geral, considerando a importância clínica da alteração e o prognóstico após a utilização do medicamento (ORUCH et al., 2014; BRASIL, 2016).

Apesar da sua eficácia o Li é contraindicado para pacientes com insuficiência renal grave, bradicardia sinusal, arritmias ventriculares graves, insuficiência cardíaca congestiva, hipotireoidismo ou hipersensibilidade ao fármaco (BRASIL, 2016). Além disso não deve ser utilizado por gestantes, devido à probabilidade de causar defeitos cardíacos no feto. Se as concentrações de Li forem superiores a 2,5 mEq/L a terapia deve ser interrompida imediatamente, devido aos eventos adversos crônicos, os quais incluem: tremor das mãos, hiperirritabilidade muscular, convulsões, hipercolesterolemia, hipertireoidismo, hiperparatireoidismo, hipercalcemia, poliúria e diabetes insipidus nefrogênico (ORUCH et al., 2014).

2.4. Interações medicamentosas

Devido à dificuldade do tratamento do TAB, o Li é utilizado em combinação com outros fármacos para reduzir o risco de episódios maníacos e os sintomas depressivos, pois um fármaco isolado não é suficientemente efetivo para todos os pacientes. Portanto, o tratamento combinado pode ser uma boa opção. Se houver prescrição médica, podem ser utilizados inibidores seletivos da recaptação de serotonina, benzodiazepínicos, anticonvulsivantes e antidepressivos (HANEMANN, 2010).

O uso simultâneo de Li com estes fármacos no tratamento do TAB resulta em interações medicamentosas, que podem reduzir ou aumentar os níveis plasmáticos de Li contribuindo ou não para a continuação do tratamento, porém a complexidade da doença e a variabilidade das características clínicas, dificultam o estabelecimento de um tratamento ideal. As interações medicamentosas com Li são diversas, envolvendo uma variada classe de fármacos como: diuréticos, antiinflamatórios não esteroidais, bloqueadores de canais de cálcio, inibidores da enzima conversora de angiotensina (IECA), antidepressivos e anticonvulsivantes. Dentre essas interações, as mais frequentes, são as interações com antagonistas da dopamina-2, como

clozapina, thiothixene, flufenazina, risperidona, olanzapina, e haloperidol, as quais potencializam a ação do Li, resultando em efeitos como: fraqueza, discinesias, encefalopatias e danos cerebrais (GOODMAN; GILMAN, 2006).

O uso concomitante do Li com diuréticos como a furosemida, pode resultar no aumento das concentrações e sintomas de toxicidade (fraqueza, tremores, sede excessiva, confusão mental). A furosemida age na alça de Henle inibindo a reabsorção de Na^+ e reduzindo a depuração de Li. Diuréticos tiazídicos como a hidroclorotiazida, também aumentam as concentrações de Li por redução da sua depuração, sendo necessário monitorar os níveis séricos de Li. Diuréticos poupadores de K^+ como amilorida e triantereno também reduzem a depuração de Li, mesmo com ação moderada, inibem a excreção de K^+ e agem diretamente no transporte tubular renal, impedindo a entrada de Na^+ nas células do túbulo distal e do ducto coletor, aumentando os níveis de Li. A espironolactona, também é um diurético poupador de potássio, que diminui a reabsorção de Na^+, cujo uso associado com Li pode tornar-se necessário ajustar a dose do mesmo. (GOODMAN; GILMAN, 2006).

Pacientes que fazem uso concomitante de Li e acetazolamida, podem ter aumento nas concentrações de Li. A acetazolamida, é um antibiótico da classe das sulfonamidas, porém atua nos rins, exercendo efeito diurético e alcalinizante na urina, promovendo a reabsorção de Na^+ e de íons de Li no túbulo proximal renal, resultando no aumento da litemia. Para alguns pacientes em terapia com Li, também pode ser prescrito o uso de carbamazepina para auxiliar no tratamento do TAB, devido à potencialização das ações do ácido gama-aminobutírico (GABA) e ao início de ação rápido. Entretanto, a carbamazepina ainda não tem um mecanismo de resposta terapêutica totalmente conhecido, pois seu uso associado com Li pode causar neurotoxicidade aditiva (fraqueza, tremores, nistagmo, asteríxis), mesmo sem alterar os parâmetros farmacocinéticos do Li (GOODMAN; GILMAN, 2006).

O uso associado de Li com antiinflamatórios não-esteroidais (AINES) pode elevar a toxicidade do Li, através da redução de sua excreção, havendo necessidade de monitorar seus níveis séricos. A interação ocorre porque os AINES exercem atividade antiinflamatória, inibindo as ciclooxigenases (COX) responsáveis pela síntese de prostaglandinas. As prostaglandinas modulam o tônus vascular e o equilíbrio hídrico dos rins, além da modulação da hemodinâmica glomerular, reabsorção de Na^+ e água nos túbulos renais e regulação da liberação de renina. Há relatos de que as prostaglandinas estimulam a secreção renal de Li e que sua inibição acarreta no aumento da reabsorção do mesmo, elevando seus níveis e induzindo sua toxicidade, porém os AINES são pouco efetivos na função renal de seres humanos normais (MARCOLIN; CANTARELLI; JUNIOR, 2004; DE OLIVEIRA et al.,

2010). Entretanto, alguns pacientes tem maior dependência das prostaglandinas renais vasodilatadoras para uma função renal adequada, por exemplo: idosos, indivíduos com insuficiência cardíaca, doenças renais prévias, diabéticos, cirróticos e hipovolêmicos (KUMMER; COELHO, 2002).

Os fármacos IECA interagem com Li, causando sua retenção e consequentemente toxicidade, cujos efeitos tóxicos incluem: fraqueza, tremores, sede excessiva, confusão mental e/ou nefrotoxicidade. Captopril, enalapril e lisinopril são exemplos desta classe de fármacos que tem interação com Li (HANEMANN, 2010). O mecanismo desta interação não foi completamente elucidado, porém alguns relatos apontam que os IECA agem impedindo a conversão da angiotensina I para angiotensina II, a qual estimula vasoconstrição e secreção de aldosterona (CUPERTINO et al., 2016). Com a inibição da aldosterona não ocorre a reabsorção de Na^{+}, acarretando no acúmulo de Li e aumento da ocorrência dos efeitos adversos (DE OLIVEIRA et al., 2010). Os antagonistas dos receptores da angiotensina II, como a losartana por exemplo, aumentam significativamente o *steady state* das concentrações plasmáticas de Li, devido ao aumento da reabsorção do mesmo, causando efeitos como: fraqueza, tremores, sede excessiva, e confusão mental (GOODMAN; GILMAN, 2006).

Outros fármacos que também apresentam interações com Li, são os bloqueadores dos canais de Ca^{2+}, como o verapamil. O uso concomitante deste fármaco com Li pode causar neurotoxicidade, perda de controle de episódios maníacos e bradicardia, devido ao aumento da sensibilidade aos efeitos do Li. Portanto, é necessário que pacientes em uso simultâneo de Li e bloqueadores de canais de Ca^{2+}, sejam monitorados rigorosamente quanto aos sinais de mania ou psicose, bem como os sintomas de neurotoxicidade como ataxia, tremores, zumbido, náuseas, vômitos ou diarreia. Esta interação tem como mecanismo o sinergismo da diminuição do transporte de íons de Ca^{2+}. A administração simultânea de Li e verapamil diminui os níveis séricos de Li, acarretando na exacerbação da psicose maníaca (HANEMANN, 2010). A diminuição do efeito do Li, está possivelmente relacionada com o bloqueio dos canais de Ca^{2+}, que atua impedindo a formação de novas sinapses, lembrando que para o Li ter efeito terapêutico, é necessário haver atividade sináptica (KIM; THAYER, 2009).

A teofilina é um antagonista não seletivo dos receptores da adenosina, cujo uso associado com Li diminui sua eficácia. A diminuição da eficácia do Li ocorre devido ao aumento de seu *clearance*, reduzindo seu tempo de meia-vida e suas concentrações séricas. Os fármacos inibidores seletivos da recaptação de serotonina (ISRS) são prescritos para o tratamento de depressão associada ou não com ansiedade. O uso combinado dos ISRS com Li pode eventualmente, aumentar os níveis de Li e/ou o risco de síndrome serotoninérgica,

caracterizada por sintomas como estado mental alterado, disfunção autonômica e anormalidades neuromusculares. Os principais fármacos pertencentes a classe dos ISRS são: fluoxetina, citalopram, fluvoxamina, sertralina e paroxetina. Alguns anti-hipertensivos como a metildopa elevam o risco de toxicidade pelo Li, em virtude do aumento da resposta do SNC ao Li. Portanto, é necessário alterar a dose de Li ou então fazer a substituição da metildopa por outro anti-hipertensivo (GOODMAN; GILMAN, 2006).

Outra interação importante, é a com bebidas alcoólicas, que devem ser consumidas somente em baixas doses, pois o álcool é uma droga depressora do SNC e exerce efeito diurético, acarretando na diminuição de sal e na alteração das concentrações de Li (NASCIMENTO et al., 2015). O Li não possui interação com benzodiazepínicos, moduladores de humor como topiramato e lamotrigina, antiparkinsonianos (levodopa), antidepressivos tricíclicos, e inibidores de monoaminaoxidase (IMAO) (HANEMANN, 2010).

2.5. Monitoramento terapêutico do Li

Os efeitos adversos estão relacionados às características farmacocinéticas dos indivíduos e às interações medicamentosas que favorecem a toxicidade, através da alteração pronunciada das concentrações eritrocitárias de Li em relação às plasmáticas, contribuindo para a baixa adesão ao tratamento (ROSA et al., 2006; BALKHI et al., 2009). Portanto, o monitoramento da exposição sistêmica ao Li, torna-se indispensável, principalmente na farmacoterapia combinada de fármacos (DUQUE et al., 2017).

O monitoramento tem a finalidade de avaliar a eficácia terapêutica e a adesão ao tratamento prescrito do ponto de vista correto de doses e intervalos, encorajando o uso do Li durante o tratamento de manutenção. As amostras devem ser coletadas 12 horas (±0,5 h) após a administração da última dose, cujo intervalo terapêutico recomendado para o Li sérico é de 0,6 a 1,2 mEq/L (BRASIL et al., 2016). A tabela 1 indica os níveis terapêuticos recomendados pelo Ministério da Saúde do Brasil, pela Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) Guidelines e pelos referidos autores no início do tratamento, durante a fase de manutenção, em episódios maníacos, hipomaníacos e depressivos e níveis tóxicos. Pacientes bipolares que fazem tratamento com Li em ambulatórios especializados com acompanhamento periódico e monitorização dos níveis séricos de Li podem ter adesão de até 85%, apresentando níveis plasmáticos de 0,6 a 1,2 mEq/L, enquanto que os não-aderentes podem ter níveis inferiores ou superiores a essa faixa terapêutica, levando às frustrações na psiquiatria (ROSA et al., 2006; DUQUE et al., 2017).

Tabela 1: Circunstâncias relacionadas às diferentes concentrações séricas de Li

Circunstâncias	**Concentrações séricas (mEq/L)**
Faixa terapêutica no início do tratamento	0,5 a 1,2 [2]
Níveis terapêuticos durante a fase de manutenção	0,5 a 1,2 [4] / 0,6 a 1,2 [1]
Níveis terapêuticos para prevenção de episódios depressivos	0,8 a 1,2 [5]
Níveis terapêuticos para episódios de mania, fase de manutenção e prevenção de hipomania	0,5 a 1,0 [3]
Aumento do risco de toxicidade	> 1,5 [1]
Efeitos tóxicos graves	> 2,0 [4]
Toxicidade crônica	> 2,5 [3]
Intoxicação caracterizada por efeitos tóxicos severos como convulsões, coma e insuficiência renal, sendo potencialmente fatal	> 3,5 [1]

Fonte: Brasil (2016); AGNP Guidelines (2018); Oruch et al. (2014); Zung et al. (2010); Severus et al. (2010) [1, 2,3,4,5]

O monitoramento periódico da litemia é recomendado nas Diretrizes Terapêuticas do Transtorno Afetivo Bipolar do tipo I (BRASIL, 2016), devendo ocorrer semestralmente no tratamento de manutenção, e serve de base para estabelecer regimes de dose individualizados. Adicionalmente, a dosagem sérica do Li tem sido utilizada como indicativo de adesão e até mesmo de eficácia terapêutica. Os ajustes de dose para fases de manutenção são feitos a partir da concentração sérica mensurada de Li em relação à dose administrada, seguindo o método

farmacocinético linear, conforme a equação 1, sendo DI dose individualizada; CLss Li sérico em equilíbrio dinâmico (nmol/L); D dose (mg) (CARSON et al., 1992).

Equação 1:

$$DI\ (mg) = (CLss\ desejada / CLss\ medida) * D\ atual$$

O monitoramento das concentrações de Li é realizado usualmente em amostras de soro. Porém, o uso de outras matrizes biológicas tem sido proposto de modo a fornecer informações adicionais sobre a terapia com o Li. A dosagem sanguínea do Li pode ser associada aos seus níveis séricos, de forma a estimar a concentração eritrocitária de Li e o índice de Li através da equação 2, onde LiR representa o índice eritrocitário de Li. Estes marcadores foram previamente associados ao desenvolvimento de eventos adversos e eficácia terapêutica, (CAMUS et al., 2003), portanto poderiam ser considerados no monitoramento terapêutico do Li. Além disso, muitas interações medicamentosas resultam em aumento ou diminuição do índice eritrocitário, às vezes, por alteração mais pronunciada dos seus níveis em relação aos plasmáticos, o que pode tornar a medida do índice de Li indispensável, principalmente quando há regimes combinados de drogas, conforme apontam estudos pré-clínicos (CAMUS et al., 2003; BALKHI et al., 2009).

Equação 2:

$$(LiR = concentração\ eritrocitária / concentração\ plasmática).$$

As concentrações eritrocitárias de Li estão relacionadas à variabilidade genética no sistema de co-transporte Na^+/Li, podendo ser determinadas por duas técnicas: a técnica direta, em que as hemácias são hemolisadas para a dosagem de Li, ou a técnica indireta pela dosagem de Li no sangue total, com hemácias hemolisadas e aplicação de um cálculo que leva em conta a concentração sérica e o hematócrito do paciente. O valor do Li nas hemácias é calculado pela equação 3, onde HCT refere-se ao hematócrito (HANEMANN, 2010).

Equação 3:

$$Li\ eritrocitário = Li\ no\ sangue\ total - (1\text{-}HCT) \times Li\ plasmático / HCT$$

2.6. Estratégias de amostragem alternativas (DBS e DPS)

O uso de manchas de sangue seco em papel (*Dried Blood Spots*) tem surgido como uma estratégia de amostragem alternativa no MTF, devido à sua praticidade na coleta e no transporte. Este método consiste em coletar uma gota de sangue em papel filtro, possibilitando coletas levemente invasivas, mantendo a estabilidade dos analitos e inativando os microrganismos possivelmente presentes na amostra, permitindo o transporte através do serviço postal convencional. No entanto, o uso de DBS possui algumas limitações, pois necessita de técnicas analíticas altamente sensíveis e aplicabilidade em grandes lotes, visto que o volume das amostras é relativamente baixo (DE LIMA et al., 2014).

Outra limitação do DBS é a influência do hematócrito na dispersão do sangue no papel, na homogeneidade da amostra e no rendimento da extração do analito. Nas amostras de pacientes com hematócrito mais elevado, a viscosidade do sangue é maior e consequentemente a dispersão do sangue no papel será menor, prejudicando a dispersão no papel, enquanto que pacientes com hematócrito mais baixo o sangue será mais fluido e terá maior dispersão no papel, reduzindo a homogeneidade da amostra e fazendo com que a concentração do analito seja possivelmente maior na periferia da mancha do que no centro. Além disso, nas amostras de DBS são medidas concentrações sanguíneas dos analitos, sendo que para a maioria dos fármacos o intervalo terapêutico é estabelecido em amostras de soro, sendo necessário o estabelecimento de estratégias para a estimativa das concentrações séricas a partir das medidas em DBS (ANTUNES, CHARÃO, LINDEN, 2016).

Outra alternativa de amostragem que também surgiu para o MTF, é o plasma seco em papel (*Dried Plasma Spots*). O uso deste método oferece vantagens sobre o método de DBS, porque além de apresentar a mesma estabilidade, a faixa terapêutica do Li é estabelecida a partir dos níveis plasmáticos, possibilitando resultados equivalentes sem a influência do efeito do hematócrito (BAIETTO et al., 2014; PARKER et al., 2015). Além disso, o volume de amostra necessário é menor, visto que a dispersão do plasma no papel é maior que a do sangue total (KOSTIC et al., 2015). As desvantagens do DPS estão relacionadas ao volume limitado de amostra, demandando de ensaios com alta sensibilidade (BAIETTO et al., 2014). Entretanto ainda não há descrição na literatura da aplicação de amostras secas para o lítio, porém o método de DBS já foi utilizado por De Lima et al. (2014) na área da psiquiatria para a determinação de anticonvulsivantes (DE LIMA et al., 2014). Portanto o uso associado das amostras de DPS e de DBS poderia ser considerado no monitoramento da terapia com o Li.

2.7. Métodos bioanalíticos para quantificação do Li

As concentrações de Li em amostras de fluidos biológicos podem ser determinadas por fotometria de emissão de chama, por eletrodo íon-seletivo, plasma indutivamente acoplado a espectrometria de emissão óptica, plasma indutivamente acoplado a espectrometria de massas ou por espectrofotometria de absorção atômica (LEAL; FERNANDES, 2002; BALKHI et al., 2009). Destaca-se entre estes, a espectrofotometria de absorção atômica como técnica com alta seletividade, sensibilidade e capacidade de análise com mínima preparação (FROES; WINDMÖLLER; SILVA, 2006), que poderia ser considerada para análise de amostras de DBS e DPS. O espectrofotômetro de absorção atômica com forno de grafite (EAAFG) é constituído por um tubo de grafite com aquecimento transversal e plataforma integrada recoberta com grafite pirolítico, corretor de fundo tipo Zeeman, amostrador automático e lâmpadas de descarga sem eletrodos (DOS SANTOS; GONÇALVES; JACOB, 2008).

A análise ocorre em etapas distintas através de um programa de temperatura do forno. Na etapa de secagem o solvente é evaporado e a matriz é destruída através da volatilização por pirólise, com eliminação dos interferentes presentes na amostra. Em seguida ocorre a atomização, que consiste na formação da nuvem atômica do analito e detecção do sinal de absorbância. Em alguns casos a etapa de pré-pirólise também pode ocorrer para aumentar a eliminação da matriz. Embora a análise seja realizada em etapas distintas, podem haver perdas do analito durante a pirólise ou interferência de concomitantes não-eliminados na atomização. Portanto é necessário realizar uma modificação química, utilizando um modificador químico que possa atuar aumentando a estabilidade térmica do analito ou a eficiência da pirólise pelo aumento da volatilização da matriz (FROES; WINDMÖLLER; da SILVA, 2006).

3. OBJETIVOS

3.1 Objetivo geral

Desenvolver e validar metodologias analíticas para dosagem de lítio em amostras de DBS e DPS.

3.2 Objetivos específicos

Aplicar a metodologia na dosagem de Li em DBS e DPS em amostras clínicas de pacientes com TAB em tratamento com Li;

Monitorar os níveis séricos de Li em pacientes com TAB em tratamento com carbonato de lítio;

Correlacionar as concentrações séricas de Li com as medidas em DBS e DPS;

Estimar as concentrações séricas de Li a partir das medidas em DBS;

Calcular o índice de lítio e as concentrações eritrocitárias a partir das obtidas em DBS e DPS.

ARTIGO 1

DETERMINATION OF LITHIUM IN DRIED BLOOD SPOTS AND DRIED PLASMA SPOTS BY GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY: METHOD DEVELOPMENT, VALIDATION AND CLINICAL APPLICATION

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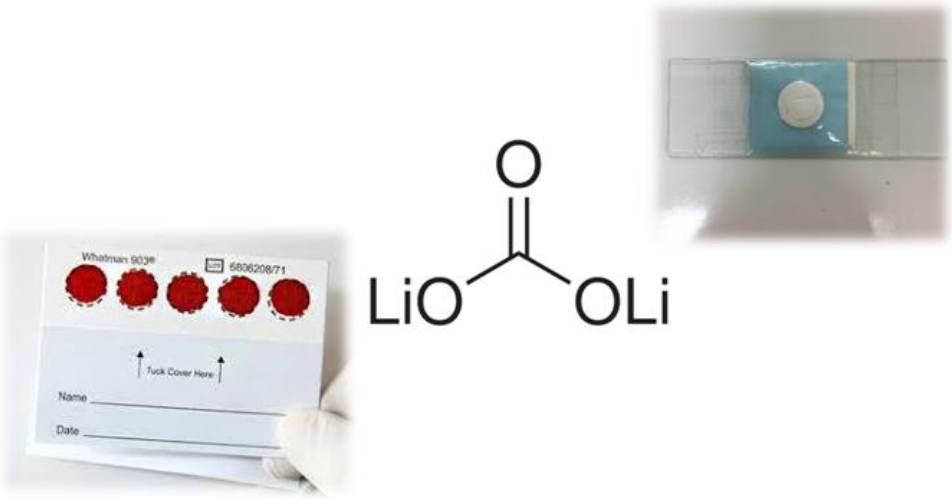

Graphical Abstract

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ABSTRACT

Due to its narrow therapeutic range, therapeutic drug monitoring (TDM) has become a standard of care for the mood stabilizer lithium (Li). Dried Blood Spots (DBS) and Dried Plasma Spots (DPS) are promising alternative sampling strategies for TDM, which allows simple and cost-effective logistics in many settings, particularly in Developing Countries. DBS and DPS are of particular interest to Li TDM for allowing for the estimation of Li erythrocyte levels. Thus, the aim of this study was to develop and validate an assay for the determination of Li in DBS and DPS by Graphite Furnace Atomic Absorption Spectrometry (GFAAS), and to evaluate its application in a clinical setting. Li was extracted from one 8 mm DBS disc punch with nitric acid 4.5% and from one 6 mm DPS disc punch with diluent solution (HNO_3 1% + Triton 0.1%) and injected into GFAAS. The method was applied to Li TDM in 43 patients with mood disorder. The assays were linear from 0.10 to 3.0 mEq/L ($r > 0.99$), precise, with CV 3.6-7.2% for DBS and 4.6-9.3% for DPS samples, and accurate 97-109% and 98-106% for DBS and DPS samples, respectively. Li was stable in dried samples during twenty days at up to 42°C. The DBS assay accuracy and recovery were not influenced by blood hematocrit. The patients presented Li serum concentrations of 0.18 to 1.1 mEq/L and 0.17 to 0.84 mEq/L in DBS and 0.15 to 0.99 mEq/L in DPS samples. DPS had comparable Li concentrations to the ones found in fresh serum samples. With DBS samples it was possible to estimate the Li ratio (LiR). The findings of this study support the clinical application of DBS and DPS samples on the TDM of Li.

Keywords: Lithium, Therapeutic Drug Monitoring, Dried Blood Spots, Dried Plasma Spots, Graphite Furnace Atomic Absorption Spectrometry, Toxicity.

1. Introduction

Lithium (Li) remains the main drug for the treatment of bipolar disorder. The effectiveness of long-term therapy with lithium salts in managing acute mood episodes, preventing relapse, and reducing suicide risk have been supported by numerous studies [1,2,3]. The initial daily dose of Li carbonate ranges from 900 to 1,200 mg and can increase up to 1,500 mg during maintenance treatment. For elderly people, with clearance lower than 80 mL per minute, the recommended dose is 300 mg twice a day [4].

The mood stabilizer is rapidly and completely absorbed from the gastrointestinal tract after ingestion, and is slowly distributed (6 to 10 hours) without binding in plasma proteins. The Li can be transported inside erythrocytes by passive diffusion, bicarbonate-influence diffusion, exchange at sodium site of the Na^+/K^+ pump, and extrusion against sodium through the sodium–lithium counter-transport (SLC) system. The inter-individual variation in the degree of Li accumulation in erythrocytes is mainly attributed to the differences in SLC activity [1, 5]. Serum peak concentration occurs 2 to 6 hours after the ingestion of an immediate release preparation of Li carbonate and is extended by 4 to 12 h for sustained-release preparation. Lithium is mainly eliminated by the kidneys as a free ion, with half-life of 16 to 29 hours in patients with normal renal function [6]. Renal Li clearance varies depending on several factors, such as age, sodium intake, total body weight, renal function, and a concomitant use of nonsteroidal anti-inflammatory drugs [1,6].

Besides its effectiveness, Li has a narrow therapeutic window and is characterized by large intra- and inter-individual variability on drug pharmacokinetic. For long-term use, concentrations of 0.5–0.8 mEq/L in blood are recommended and for an acute treatment an increase of concentrations up to 1.2 mEq/L can be justified [2]. Neurotoxic events, hyperthyroidisms, hypercalcemia, among others are reported in concentrations above 2.5 mEq/L [2,7,8]. Also, treatment nonadherence is a persistent problem in psychiatric, approximately 54% of the patients not adhere to medical prescription [5], which has been associated with side effects occurrence, lack of illness knowledge, complex treatment regimen, and polytherapy [4,9].

Controlled clinical trials have shown beneficial effects of Li Therapeutic Drug Monitoring (TDM), which allows for dose adjustment, avoiding the occurrence of toxicity [6]. Therefore, the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) consensus Guidelines for TDM in psychiatry strongly recommends the measurement of Li trough serum levels [2]. Besides serum, measuring drug concentrations in whole blood

have been suggested to provide additional information about Li therapy. Li blood concentrations could be associated to serum levels, to estimate the erythrocyte concentration and, subsequently, the erythrocyte to plasma concentration ratio (LiR). These biomarkers were previously associated with side effects occurrence and therapeutic effectiveness [5], and could be considered during Li TDM. Furthermore, many drug interactions result in increase or decrease of the Li erythrocyte ratio, sometimes for more pronounced alteration of erythrocyte levels in relation to plasma levels [1,5,10].

The Dried Blood Spots (DBS) has arisen as an alternative sampling strategy in TDM due its convenience in transport and collection. The method consists in collecting a blood drop from finger prick onto a standardized filter paper card. Typically, the analytes are more stable in the dried matrix, with no enzymatic reactions, and microorganisms are inactivated, allowing transport through conventional postal service [11]. Also, DBS allows the possibility of training patients to take their own samples at collection times adequate to their personal posology [12]. Dried plasma spots (DPS) can also be obtaining from finger prick with the use of membranes that filter the erythrocytes. DPS samples offer advantages over DBS, with the same stability, as it is supposed to be representative of serum levels [13]. However, the volume of sample recovery is lower, since plasma dispersion in paper is higher than total blood [14].

The use of dried blood and plasma samples in psychiatric have been proposed [15, 16]. However, to the best of our knowledge, there is no report a method for monitoring Li levels in DBS or DPS samples. Therefore, the aim of this study was to develop a method for the determination of Li in DBS and DPS by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) with Zeeman background correction, and correlate with serum concentrations to evaluate the clinical application in patients with bipolar disorder.

2. Material and methods

2.1. Standards, solvents and materials

The Li stock solution (144 mEq/L) was acquired from Perkin Elmer® (Shelton, USA), nitric acid (HNO_3) 65% from Merck Millipore Corporation® (Darmstadt, Germany), and Triton X-100 solution was obtained from Sigma-Aldrich® (Saint Louis, USA). The water was purified in a Purelab Elga® system from Veolia Labwater (High Wycombe, UK). Whatman 903® paper was obtained from GE Healthcare (Westborough, USA). The DPS collection device was made

by the researchers with a nanostructured polsylfone membrane on top of the whatman 903® filter card. Graphite tubes were purchased from Perkin Elmer® (Shelton, USA).

2.2. Solutions and validation samples

All glassware were washed thoroughly with detergent solutions, rinsed with water, placed in a nitric acid 10% (v/v) bath for overnight, and finally, prior to use, rinsed several times with ultrapured water. The autosampler cups were submitted to the same treatment. The diluent solution 1% HNO_3 and 0.1% Triton X-100 was prepared diluting HNO_3 65% and Triton X-100 in ultrapure water. The extraction solution was HNO_3 4.5% in ultrapure water. Working standard solutions were prepared by diluting the Li stock solution with diluent at different concentrations 2, 6, 12, 18, 44, and 60 mEq/L. Blank blood samples used for the preparation of calibration and quality control samples, were obtained from healthy volunteers. Calibration and quality control (QC) samples were prepared diluting the Li working solutions with venous blood, with Hct 40%, or plasma in the proportion of 1:20 (v/v). After, 50 µL of the CQ samples prepared in blood, and 20 µL CQ samples prepared in plasma were pipetted on the Whatman 903® paper to obtain DBS and DPS samples, respectively. All CQ samples were dried for at least 3 hours at room temperature.

2.3. Optimization of Li extraction from DBS and DPS samples

The liquid extraction of Li from DBS samples was optimized by response-surface analysis, using a Box-Behnken experimental design. The experimental factors evaluated in the experiment were: extraction solvent composition (HNO_3 at 2, 4.5 and 7%); extraction time (30, 105 and 180 minutes) and extraction temperature (25, 47 and 70°C). The extracted were further diluted 5 times with the diluent solution. The optimized condition was calculated based on the desirability function, aiming to maximize the Li peak area. Evaluation of experimental data was performed using Design Expert 11® (Stat-Ease, USA). These same conditions were selected for DPS samples, but diluent solution was employed as extraction solvent.

2.4. DBS and DPS sample preparation

One 8 mm DBS disk was transferred to a 2 mL polypropylene microtube, followed by the addition of 1 mL of nitric acid 4.5%. The sample was incubated at 25°C for 30 min at 750 rpm in a ThermoMixer® (Eppendorf). An aliquot of 200 µL from the supernatant was transferred to autosampler cups containing 800 µL of diluent solution and 20 µL injected into the graphite furnace atomic absorption spectrometer (GFAAS).

Considering the size of the plasma spot recovered through the DPS disposal, smaller disks were used for this sample extraction. One 6 mm disk was transferred to a 2 mL polypropylene microtube, followed by the addition of 1 mL of diluent solution. The sample was incubated at 25°C for 30 min at 750 rpm in a ThermoMixer® (Eppendorf). An aliquot of 900 µL from the supernatant was transferred to autosampler cups and 20 µL injected into the GFAAS.

2.5. Equipment and analysis conditions

A PinAAcle 900Z graphite furnace with AS 900 Autosampler and AAnalyst 900 atomic absorption spectrometer equipped with a transversely heated graphite atomizer, longitudinal Zeeman effect background corrector, and a Li hollow cathode lamp, all from Perkin Elmer® (Shelton, USA) was used in the analysis. An aliquot of 20 µl of the DBS and DPS extracts were injected in the system. The graphite furnace heating program, comprising drying, pyrolysis, atomization and cleaning steps were as described in Table 1. The absorbances were monitored at 670.8 nm, with argon as protection gas

Table 1- Graphite furnace heating program.

Process	Temperature (°C)	Ramp time (seconds)	Temperature time (seconds)	Internal flow (mL/min)
Drying	110	2	5	250
	130	5	5	250
Pyrolysis	900	20	20	250
Atomization	2200	0	6	0
Cleaning	2450	1	3	250

2.6. Linearity

Linearity assay was carried out through DBS and DPS extraction containing 0.10, 0.3, 0.6, 0.9, 1.4, 2.2 e 3.0 mEq/L and analyzed in sextuplicate. Calibration curves were fitted through linear equation obtained onwards samples absorbance values compared with calibrators absorbance values. Homoscedasticity of calibration data was evaluated with F-test at the confidence level of 95%. Weighted least-squares linear regression was used to generate calibration models, which were assessed through their coefficients of correlation (r) and cumulative percentage relative error (Σ%RE).

2.7. Precision and accuracy

Blank DBS and DPS samples were enriched with Li and pipetted to paper Whatman 903® to obtain quality controls (QC) at the concentration levels of 0.25 mEq/L (quality control at low concentration, QCL), 1.5 (quality control at medium concentration, QCM), 2.4 (quality control at high concentration, QCH). QC samples were analyzed in triplicate on five different days. Intra-assay precision and inter-assay precision were calculated by one-way ANOVA with the grouping variable "day" and were expressed as CV%. Accuracy was defined as the percentage of the nominal concentration represented by the concentration obtained with the calibration curve. The acceptance criteria for accuracy were mean values within ±15% of the theoretical value, and for precision, a maximum CV of 15% was accepted [17].

2.8. Selectivity

Blank DBS and DPS samples were obtained from six different human sources non-users of Li and were extracted and analyzed as described in "*DBS and DPS sample preparation*" to verify the presence of absorbance peaks that might interfere in Li detection.

2.9. Sensitivity

Precision and accuracy were also assessed at the concentration level of the lowest calibrator, 0.10 mEq/L (Quality control at the lower limit of quantification, QCLOQ), being tested in triplicate on three different days for both DBS and DPS samples. The acceptance criteria established for the limit of quantification was accuracy within 100 ± 20% of the nominal concentration and a maximum CV% of 20 [17].

2.10. DBS and DPS extraction yield

Aliquots of 18 µl of blood (Hct% 40) for DBS and 7 µl of plasma for DPS containing Li at the concentrations of QCL, QCM and QCH were added to Whatman 903® paper. Whole spots were extracted in triplicate as described previously. Li solutions in concentrations equivalent to 100% extraction yield were also analyzed. The extraction yield was calculated comparing the areas of Li in extracted QC and solution samples.

2.11. Stability at DBS and DPS stored at different temperatures

Thermal stability was assessment in DBS and DPS samples at QCL and QCH concentrations, stored at -20, 25 and 42°C and analyzed in triplicate 3, 6, 9, 15 and 20 days after spotting on the paper. Stability was considered acceptable if all results were within the range of 85-115% of the concentrations measured at the beginning of the series.

2.12. Benchtop stability

The stability of processed DBS and DPS samples kept on the GFAAS autosampler was tested in QCL and QCH samples. The samples were extracted and analyzed at time intervals of 1 hour during 3 hours. Tendencies of instability for identify by regression analysis plotting absolute peak areas at each concentration versus injection time. A decrease or an increase of up to 15% in the measured peak areas through the time was considered as acceptable [17].

2.13. DBS specific validation: influence of Hct on accuracy

Aliquots of blood with different Hct levels (25, 45 and 55%) were prepared by centrifuging EDTA tubes containing blank blood and then adding or removing appropriate volumes of plasma. Li was added to these aliquots of blank blood to reach the concentrations of QCL, QCM and QCH, and 50 µL were applied onto Whatman 903®, dried at room temperature for 3 hours. The samples were analyzed in triplicate for each concentration level and Hct% value. The influence of the Hct% on Li measurement accuracy was determined as the percentages of nominal concentrations that were measured in the DBS samples. Acceptance criteria were values in the range of 85-115% [12].

2.14. DBS specific validation: influence of Hct in extraction yield

Aliquots of 18 µL of blood with Hct% 25, 45 and 55, respectively, containing Li at concentrations of QCL, QCM and QCH and non-spiked blood were pipetted onto Whatman 903® paper and dried at room temperature for 3 hours. The whole spots were cut and extracted in triplicate. Extracts from non-spiked blood were enriched with Li to achieve final concentrations equivalent to 100% extraction yield. The extraction yield was calculated comparing the areas of Li in control and non-spiked samples.

2.15. DBS specific validation: Impact of spotted blood volume on accuracy

Different volumes (30, 40 and 60 µL) of QCL and QCH blood samples, with Hct 40%, were pipetted onto the Whatman 903® paper. After drying, the DBS samples were analyzed in triplicate as described in "*DBS and DPS sample preparation*" and quantified with a calibration curve prepared after pipetting 50 µl of the blood to the paper. The impact of spotted blood volume on Li quantification was determined as the percentages of the nominal concentrations that were measured in the DBS samples. The acceptance criterion was a maximum deviation of ± 15% from nominal concentrations [12].

2.16. Method application

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee at Universidade Feevale (report number 2231794). A total of 43 volunteer patients from Center of Psychosocial Attention and Basic Health Unit, over 18 years on Li carbonate therapy for at least one week were enrolled in the study with informed consent. Data on gender, age, body mass index (BMI), smoking and alcohol consumption, adverse events, living habits and concomitant use of other drugs related to Li therapy, dose, and the duration of Li therapy were reported by patients through a questioner. Blood samples were taken 11-13 h after the last Li carbonate intake.

Capillary blood was collected after finger prick using a 2.0 mm penetration, 0.8 mm blade contact-activating lancet (Medlance®). One blood spot was collected onto the Whatman 903 DBS card and other blood spot on the disposal for DPS collection, and allowed to dry for at least 3 h at room temperature. Analyses were performed within 14 days. Venous blood was simultaneously collected within an interval of ± 5 min by venipuncture into one EDTA

containing tube and one tube with clot activator. The EDTA tube was used for Hct% determination, for obtaining venous blood DBS by pipetting one 50 µL aliquot onto the whatman 903 card, and after centrifugation obtaining one Dried Plasma Spot applied to the paper (DPAP) sample, by adding 20 µL of plasma onto the Whatman 903 card. The tube with clot activator was centrifuged and the serum used for the measurement of creatinine, TSH and Li concentrations.

Serum Li concentrations were measured after two step 10 times dilution with diluent and analyzed under the same analytical conditions as used for the dried samples, but with lower injection volume (10 µL). The method was linear from 0.10 to 3.0 mEq/L ($r>0.99$), precise (CV% 4.05 - 9.0%) and accurate (89-105%). Lithium erythrocyte concentrations were calculated by the equation *Erythrocyte Li = Li in DBS – [(1-HCT) x serum Li] / HCT*

2.17. Statistical analysis

Initially, a descriptive analysis of the study variables was conducted. Pearson correlation analysis of quantitative variables were performed. Estimated Serum Concentration (ESC) was calculated using the equation: $ESC_{Hct} = (DBS_{conc}/[1-(Hct/100)]) \times f_p$ where DBS_{conc} is the concentration measured in DBS, Hct is the individual hematocrit of patient and f_p is the fraction of the drug in plasma, according to Antunes *et al* [12]. The f_p value was adjusted to obtain a mean ratio between the measured Li serum concentrations and ESC of 1, using the above described equation. In a second approach, Estimated Plasma Concentration (EPC) was calculated using a Correction Factor (CF) based on the mean ratio of the plasma to DBS levels, without considering the individual Hct value ($ESC_{correction\ factor} = DBS_{conc} \times CF$).

The agreement between Li concentrations in serum and DPS, DPAP or ESC, and venous DBS and capillary DBS samples were evaluated using Passing-Bablok regression analysis, with 95% confidence intervals (95% CI) for slope and intercept. Bland-Altman plots were used to assess the relative differences between the two methods by plotting the percentage differences against the mean analyte concentrations from both assays. The mean relative differences and the 1.96 standard deviations (SD) of the differences were calculated and within 1.96 SD were considered acceptable [18]. Statistical analyses were performed with Medcalc® software (Ostend, Belgium).

3. Results and discussion

3.1. Results from optimization of experimental conditions

Response surface analysis was used to evaluate the results of the Box-Behnken experiment. The model describing Li peak area ratio from the process variables was not significant (P=0.089), showing little impact of the investigated variables. The only factor presenting statistical significance was the concentration of nitric acid, which was optimum from 4.5%. Based on these observations, as we intend to have the maximum area with the shorter extraction time, we selected the following extraction conditions: nitric acid at 4.5%, incubation temperature 25°C and time: 30 minutes, due to lower extraction temperature and time incubation. Same conditions were used for DPS and DPAP samples extractions.

3.2. DBS and DPS methods validation

No interfering compounds were identified in the 6 DBS or DPS samples process free Li. Calibration data exhibited significant heteroscedasticity, with F_{exp} of 95.1 for DBS and 112.0 for DPS (F_{tab} (6;5;0.95)=4.95). The regression using the weighting factor 1/x offered the best ∑%RE of the models tested, with values of -5.1×10^{-14} for DPS and 0.0 for DBS. This weighting factor was used for all subsequent validation tests and for analysis of the clinical samples. The coefficients of correlation were above 0.99 for all weighting factors.

Results of the precision and accuracy assays met acceptance criteria and are show in Table 2. Intra-assay and inter-assay imprecision ranged from 3.57 to 7.16% for DBS and from 4.59 to 9.25% for DPS, demonstrating repeatability of the methods. Accuracy was within 96.5% to 108.8% for DBS and 97.7% to 105.6% for DPS. The methods presented adequate sensitivity, the QCLLOQ samples at 0.10 mEq/L presented acceptable imprecision, CV between 7.01 and 12.07%, and accuracy from 90.6 to 97.5%. Li concentrations in biological fluids can be measured by several methods as flame emission photometry (FES), by ion-selective electrode (IES), inductively coupled plasma mass spectrometry (ICP-MS) or by atomic absorption spectrometry [1]. The atomic absorption spectrometry with graphite furnace stands out as an analytical technique with high selectivity and sensitivity [19] that allowed the accurate and precise measurement of Li levels in small sample volume, as DBS and DPS.

Table 2- Precision, accuracy, sensitivity and extraction yield validation tests in DBS and DPS samples

QC Sample	Concentration (mEq/L)	Precision (CV %)		Accuracy (%)	Extraction yield (%)
		Intra-assay	Inter-assay		
Dried Blood Spot					
QCLOQ	0.10	11.0	14.0	104.0	-
QCL	0.25	5.45	7.16	96.5	75.6
QCM	1.5	5.24	3.57	101.5	81.0
QCH	2.4	4.22	5.15	108.8	83.3
Dried Plasma Spot					
QCLOQ	0.10	20.0	18.0	113.0	-
QCL	0.25	4.59	9.25	104.7	47.6
QCM	1.5	8.80	6.93	97.7	45.0
QCH	2.4	9.31	5.97	105.6	56.3

QCLOQ: Quality Control on Limit of Quantification, QCL: Quality Control Low, QCM: Quality Control Medium, QCH: Quality Control High

Whatman 903® paper was chosen due to its availability, low purchase cost, and a high standardization degree [12]. Sample preparation was simple, based on liquid extraction. Mean extraction yield was 79.9% for DBS and 49.6% for DPS samples. Besides the lower recovery in DPS samples, the method presented satisfactory sensitivity considering the extent of concentrations in clinical samples.

Stability of processed samples at room temperature was acceptable, once Li concentration changes after 3 hours ranged from +2.80 to +7.7% in DBS extracts and -8.0% to 10% in DPS extracts, indicating that large analytical batches can be processed without the need of refrigeration to preserve extracts.

Information about the thermal stability of the analytes in dried samples is needed, as samples can be exposed to high temperatures when transported by regular mail [20]. To the best of our knowledge, until now there was no data in the literature assessing the stability of Li in dried samples. We tested the drug stability in DBS and DPS samples at QCL and QCH

concentrations, maintained at -20, 25 and 42 °C during twenty days as demonstrated in Table 3. These temperatures were selected to simulate conditions that samples sent by post could potentially be exposed, also considering the possible need of refrigeration. No significant changes were observed in Li concentrations neither DBS nor DPS samples stored over a period of 20 days at the three tested temperatures, varying from 87 to 110% for DBS and 86 to 113% for DPS in relation to the first day analyzed. These results suggest that the dried samples do not require refrigeration, allowing transport through postal conventional service. Other studies already reported Li stability in fresh serum at different storage conditions. Guilherme (2007) related Li stability in fresh serum at different temperatures (-20 and 25°C) short-term (21 hours), three freeze/thaw cycles at 12 hours and long-term (248 days) presenting values of 88% to 115% upon the first analysis [21].

Table 3 – Long-term Li stability at DBS and DPS samples

QC sample	Concentration (mEq/L)	Temperature (°C)	Day 9 (%)	Day 15 (%)	Day 20 (%)
Dried Blood Spot					
QCL	0.25	-20	90	96	87
		25	95	97	97
		42	101	92	89
QCH	2.4	-20	93	91	110
		25	94	90	110
		42	93	90	107
Dried Plasma Spot					
QCL	0.25	-20	109	108	113
		25	104	98	111
		42	100	86	92
QCH	2.4	-20	108	110	109
		25	100	101	104
		42	92	99	99

QCL: Quality Control Low, QCH: Quality Control High.

3.3. DBS specific validation

Once the blood viscosity varies according to the Hct, the sample volume in a fixed diameter spot and the extraction yield may be influenced by this variable and need tested. Lithium measurements in DBS prepared with Hct 25, 45 and 55% whole blood samples resulted in concentrations in the range of 85 and 115% from the nominal value. Lower accuracy was found for QC samples prepared with Hct 25%, as a result of the lower spotted volume in the 8 mm DBS disk. On the other hand, the highest value was observed in QC samples with Hct 55%, due to the larger spotted blood volume. On this basis, analytical curves prepared with Hct 40% blood cover most of patients Hct% range, from 25 to 55%. Besides meeting the criteria for acceptance, the accuracy could be improved preparing the calibration curve with blood Hct value closer to patient's samples, or correcting the result with the blood volume in the 8 mm diammeter for each Hct value (15, 18 and 20 µl for Hct 25, 45 and 55%, respectively). With the last approach, accuracy values improved to 92 to 105% without proportional differences between different Hct. Patients Hct can be addressed, among others, measuring the intracelular ion potassium (K+) levels in the DBS sample [22]. The extraction yield was not affected by the Hct either, as the extent of Li recovery was consistent throughout the tested range, but it was moderately affected by drug concentration, being moderately higher at high concentrations, as shown in Table 4. However, it does not impact significantly on the assay accuracy, since the calibrator's samples comprise a wide range of concentrations.

Table 4 – Impact of Hct DBS method accuracy and extraction yield

Hct (%)	QC sample	Nominal concentration (mEq/L)	Accuracy (%)	Accuracy improved by volume Hct (%)	Extraction yield (%)
	QCL	0.25	85	102	83
25	QCM	1.5	86	103	84
	QCH	2.4	85	102	90
	QCL	0.25	92	92	83
45	QCM	1.5	94	94	83
	QCH	2.4	105	105	93
	QCL	0.25	106	95	82
55	QCM	1.5	113	101	82
	QCH	2.4	115	104	90

QCL: Quality Control Low, QCM: Quality Control Medium, QCH: Quality Control High.

All DBS calibration curves were prepared pipetting 50 µL of spiked blood onto the Whatman 903® paper. The influence of the spotted volume on method accuracy was tested to simulate volume variations of capillary blood drops collected from patients. The volume of the blood drop, from 30 to 60 µL, did not impact on assay accuracy, as demonstrated in table 5.

Table 5 – Influence of spotted volume on accuracy of Li concentrations

Volume (µL)	QC sample	Nominal concentration (mEq/L)	Accuracy (%)
30	QCL	0.25	98
	QCH	2.40	88
40	QCL	0.25	89
	QCH	2.40	112
60	QCL	0.25	107
	QCH	2.40	110

QCL: Quality Control Low, QCH: Quality Control High.

3.4. Clinical application

Patient's clinical and demographic characteristics are listed in Table 6. Most of the individuals were women (69.7%), Li carbonate doses ranged from 300 to 1,200 mg daily, hand tremors was the most reported averse event, followed by fatigue and increase of body weight. Serum creatinine were within 0.31-1.36 mg/dL (guideline values: 0.6-1.2 for women and 0.7-1.3 for men) and TSH from 0.50-14.72 µUI/mL (guideline values: 0.4-4.5).

Table 6– Characteristics of patients and side effects (n=43)

Characteristic	n=43
Gender (Men/women)	13/30
Age (years) (mean ± SD)	46.51 ± 14.10
Body mass index (Kg/m^2) (mean ± SD)	26.29 ± 4.24
Smoking (n)	15
Alcohol consumption (n)	4
Concomitant administration of other drugs (n)	42
Lithium carbonate treatment duration (years) (mean ± SD)	6.73 ± 6.31
Hct (%) (mean ± SD)	38.91 ± 2.75
Creatinine (mg/dL) (mean ± SD)	0.90 ± 0.18
TSH (µUI/mL) (mean ± SD)	3.30 ± 2.77
Side effects (n)	**Absent/Mild-Moderate/Severe**
Tremor hands	08/18/17
Feet or hand pain	21/15/07
Diarrhea	33/08/02
Nausea/Vomit	25/14/04
Dry skin	16/22/05
Fatigue/Exhaustion	12/12/19
Headaches	16/15/12
Discomfort or abdominal pain	29/08/06
Metallic taste	25/11/07
Weight gain	13/19/11
Rash on the skin	32/09/02

SD: Standard Deviation

Measured Li concentrations are displayed in Table 7. Li serum levels were from 0.18 to 1.10 mEq/L, Li in capillary DBS from 0.17 to 0.84 mEq/L and Li in DPS from 0.15 to 0.99 mEq/L. The Li concentrations found in DPS, DPAP and serum were significant correlated. The same degree of correlation was found between Li in serum and DPS and DPAP samples (r=0.866 and r=0.870, respectively), indicating that the plasma levels obtained from the device and applied onto the paper after blood centrifugation had equivalent results. Serum and capillary DBS had lower correlation coefficient, of r=0.734, whereas capillary and venous DBS were compared with r=0.964.

Serum Li concentrations were below the recommended therapeutic window (0.50-1.2 mEq/L) in 39.5% of the patients, suggesting potential sub dosages or adherence issues. The variability on Li serum levels could be related to individual characteristics such as renal clearance, salt ingestion, alcoholic beverages consumption and concomitant administration of other drugs, as nonsteroidal anti-inflammatory drugs [6]. In this study, almost patients presented Li concomitant administration with benzodiazepines, antidepressants and antipsychotic drugs such as risperidone, haloperidol, amitriptyline, fluoxetine, clonazepam and clozapine. None of the patients had Li serum levels above the therapeutic range, thus it was not possible to investigate the relation between toxicity and serum levels.

Conventional serum measurement of Li levels is implemented in clinical practice. The blood measurement could be used as complementary tool, allowing the estimation of erythrocyte concentrations and LiR [1,5]. Some authors reported that LiR is a better indicator of neurotoxicity than serum Li concentrations. A threshold of 0.5 to identify patients prone to toxicity have been proposed [1,5,10]. Patients LiR were estimated from DBS and DPS Li measurements (Table 8.). A high number of patients presented LiR above 0.5 (n=20), however no statistical significant association between the presence of severe adverse events and LiR classification.

Table 7 – Li concentrations in patients with bipolar disorder (n=43)

Li serum (mEq/L)	Li DPS (mEq/L)	[DPS]/ [serum] (%)	Li Venous DBS (mEq/L)	Li Capillary DBS (mEq/L)	[capillary] / [venous DBS] (%)	HCT (%)	Li erythrocytes (mEq/L)	LiR
0.46	0.37	80	0.64	0.51	80	40.2	0.04	0.09
0.53	0.37	70	0.35	0.35	100	38.2	0.16	0.32
0.58	0.47	81	0.35	0.42	120	39.9	0.31	0.64
0.61	0.6	98	0.53	0.52	98	39.6	0.28	0.41
0.46	0.44	96	0.29	0.36	124	36.1	0.03	0.05
0.52	0.56	108	0.84	0.92	110	39.9	1.06	1.27
0.34	0.4	118	0.25	0.29	116	39.1	0.18	0.50
0.49	0.52	106	0.27	0.42	156	35.6	0.29	0.60
0.31	0.23	74	0.19	0.23	121	33.1	0.15	0.55
0.75	0.7	93	0.7	0.74	106	41.5	0.6	0.71
0.76	0.82	108	0.41	0.46	112	42.9	0	0.00
0.80	0.63	79	0.69	0.71	103	44.1	0.72	1.03
0.32	0.3	94	0.3	0.27	90	35.4	0.31	1.23
0.18	0.15	83	0.17	0.17	100	38.4	0.23	1.8
0.51	0.39	76	0.26	0.33	127	38.1	0.27	0.72
1.02	0.99	97	0.83	0.88	106	37.3	0.54	0.50
0.34	0.25	74	0.2	0.22	110	40.4	0	0.0

Li serum (mEq/L)	Li DPS (mEq/L)	[DPS]/ [serum] (%)	Li Venous DBS (mEq/L)	Li Capillary DBS (mEq/L)	[capillary] / [venous DBS] (%)	HCT (%)	Li erythrocytes (mEq/L)	LiR
1.10	0.81	74	0.71	0.70	99	41.5	0.25	0.24
0.77	0.7	91	0.7	0.81	116	36.7	0.98	1.38
0.28	0.27	96	0.2	0.25	125	41.3	0.29	1.33
0.30	0.24	80	0.2	0.23	115	41.3	0.23	1.00
0.36	0.37	103	0.25	0.32	128	39.4	0.26	0.72
0.37	0.4	108	0.33	0.35	106	40.9	0.31	0.81
0.58	0.43	74	0.33	0.35	106	36.9	0.26	0.66
0.68	0.51	75	0.4	0.43	108	35.4	0.28	0.56
1.01	0.82	81	0.49	0.47	96	37.8	0.01	0.01
0.77	0.63	82	0.38	0.39	103	38.0	0.01	0.02
0.85	0.61	72	0.45	0.44	98	38.6	0.14	0.22
0.45	0.33	73	0.23	0.28	122	35.6	0.21	0.65
0.72	0.52	72	0.38	0.41	108	43.3	0.21	0.38
0.72	0.57	79	0.36	0.39	108	36.4	0.06	0.10
0.65	0.54	83	0.26	0.33	127	43.1	0.07	0.12
0.48	0.43	90	0.23	0.26	113	36.5	0.00	0.0
0.50	0.59	118	0.52	0.54	104	43.4	0.53	0.96
0.48	0.46	96	0.52	0.52	100	40.1	0.49	0.91

	Li serum (mEq/L)	Li DPS (mEq/L)	[DPS]/ [serum] (%)	Li Venous DBS (mEq/L)	Li Capillary DBS (mEq/L)	[capillary] / [venous DBS] (%)	HCT (%)	Li erythrocytes (mEq/L)	LiR
	0.28	0.2	71	0.31	0.37	119	37.7	0.54	1.98
	0.58	0.56	97	0.47	0.42	89	38.4	0.21	0.38
	0.43	0.26	60	0.22	0.23	105	43.8	0.14	0.47
	0.53	0.43	81	0.38	0.35	92	37.6	0.18	0.41
	0.52	0.52	100	0.19	0.19	100	37.1	0.17	0.42
	0.62	0.44	71	0.34	0.36	106	40.9	0.06	0.09
	0.62	0.67	108	0.61	0.56	92	37.2	0.12	0.12
	0.56	0.34	61	0.4	0.43	108	45.6	0.24	0.41
Mean	0.56	0.47	84.5	0.4	0.42	108.57	39.2	0.26	0.56
SD	0.21	0.19	14.90	0.18	0.18	13.41	2.8	0.25	0.5

DPS: Dried Plasma Spot; DBS: Dried Blood Spot; HCT: hematocrit; LiR:lithium erythrocyte to plasma ratio; SD: Standard deviation

Passing-Bablok regression (n=43) of the samples indicated no constant or proportional errors between DPS, DPAP and serum. Li in serum versus Li DPS: intercept 95% CI of -0.18 to 0.03, and slope 95% CI of 0.77 to 1.15. Li in serum versus Li DPAP: intercept 95% CI of -0.16 to 0.03, and slope 95% 0.83 to 1.24. Li concentrations were compared in DPS, DPAP with serum levels by Bland-Altman plots. The mean relative differences among Li serum concentrations and DPAP was 7.8% with three Li measurements (6.97%). Similarly, the mean difference for DPS and serum Li was 9.1%, with three Li measurements (6.97%) outside of the ± 1.96 SD (Figure 1). Cusum test P values for all comparisons were >0.05, indicating no significant difference from linearity. Furthermore, the differences were spread randomly around the means, indicating no systematic error for any evaluation. Therefore this matrices could be used to enlarge TDM of Li, allowing obtain more robust results in therapeutic efficacy evaluation.

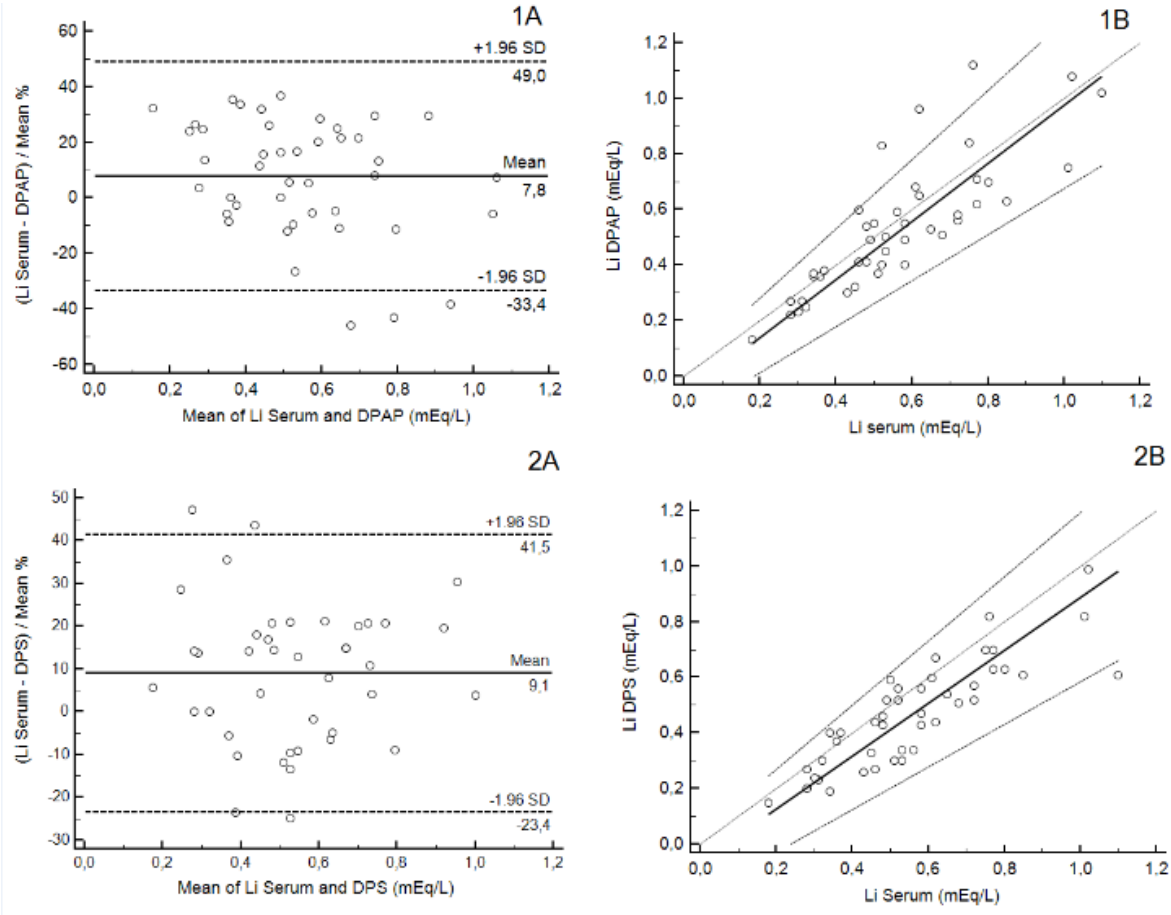

Figure 1: Bland-Altman (BA) and Passing-Bablok (PB) comparison of Li concentrations measured in serum, DPAP and DPS. 1A: BA regression serum vs. DPAP, 1B: PB plot serum vs. DPAP, 2A: BA regression serum vs. DPS, 2B: PB plot serum vs. DPS.

In an attempt to estimate serum concentrations from DBS measurements we used two approaches to calculate serum levels. Estimated serum concentrations (ESC) were calculated from capillary DBS Li measurements using individual Hct level and fp mean value of 0.82. Using this approach, mean ESC at samples was 0.57 mEq/L, represented in average 98% of the measured Li serum concentrations. Alternatively, using the correction factor without patients Hct, mean ESC was 0.56, representing in average 100% of the measured Li serum concentrations. Besides the approximation of data to the mean, there was a large dispersion of the results over the mean with a 1.96 SD range close to 60%. Thus, the use of DBS to estimate serum levels was not realible.

Li measurements in DBS and DPS could be more attractive on monitoring, due your collect and transport convenience. This first report indicate the possibility of using the dried samples for Li TDM. Further studies with larger number of patients are need to evaluate the relation of the LiR with toxicity.

4. Conclusion

Analytical method was developed and validated for Li determination in DBS and DPS samples, using GFAAS was satisfactory, considering the need of a sensitive technique, specific and with minimal preparation compared other techniques. DPS had comparable Li concentrations to the ones found in fresh serum samples, despite the low extraction yield. With DBS samples it was possible to estimate the LiR, but was not effective to estimate Li serum concentrations. The findings of this study support the clinical application of DBS and DPS samples on the TDM of Li.

5. *Acknowledgements*

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References

1. S.El Balkhi, B. Megarbane, J. Poupon, F. Baud, M. Galliot-guilley, Lithium poisoning: Is determination of the red blood cell lithium concentration useful?, Clin. Toxicol. 47 (2009) 8–13.
2. C. Hiemke, N. Bergemann, H. W. Clement, A. Conca, J. Deckert, K. Domschke, G. Eckermann, K. Egberts, M. Gerlach, C. Greiner, G. Gründer, E. Haen, U. Havemann-Reinecke, G. Hefner, R. Helmer, G. Janssen, E. Jaquenoud, G. Laux, T. Messer, R. Mössner, M. J. Müller, M. Paulzen, B. Pfuhlmann, P. Riederer, A. Saria, B. Schoppek, G. Schoretsanitis, M. Schwarz, M. Silva Gracia, B. Stegmann, W. Steimer, J. C. Stingl, M. Uhr, S. Ulrich, S. Unterecker, R. Waschgler, G. Zernig, G. Zurek, P. Baumann, Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017, Pharmacopsychiatry, 51 (2018) 9-62.
3. E. Leclercab, R.B. Mansurab, E.Brietzke, Determinants of adherence to treatment in bipolar disorder: A comprehensive review, J. Affectiv. Disord., 149 (2013) 247-252.
4. M.E.M. Duque, C. Alzate-Carvajal, K. Zapata-Castañeda, J.E. Machado-Alba, Profile of lithium carbonate use in patients with bipolar disorder in Colombia, Biomedical, 37 (2017) 43-50.
5. M. Camus, G. Henneré, G. Baron, G. Peytavin, L. Massias, F. Mentré, R. Farinotti, Comparison of lithium concentrations in red blood cells and plasma in samples collected for TDM, acute toxicity, or acute-on-chronic toxicity, Eur. J. Clin. Pharmacol., 59 (2003) 583-587.
6. C. Couffignal, L. Chevillard, S.E. Balkhi, S. Cisternino, X. Declèves, The Pharmacokinetics of Lithium. In: Malhi G., Masson M., Bellivier F. (eds) The Science and Practice of Lithium Therapy. Springer, Cham, 2017, pp. 22-53.

7. R. Oruch, M. Elderbi, H. Khattab, I. Pryme, A. Lund, Lithium: A review of pharmacology, clinical uses, and toxicity, Eur. J. Pharmacol., 740 (2014) 464–473.

8. P. Sienaert, I. Geeraerts, S. Wyckaert, How to initiate lithium therapy: a systematic review of dose estimation and level prediction methods, J. Affectiv. Disord., 146 (2013) 15–33.

9. Martha Sajatovic M.D.Marcia Valenstein M.D., M.S.Frederick Blow Ph.D.Dara Ganoczy M.P.H.Rosalinda Ignacio M.S, Treatment Adherence With Lithium and Anticonvulsant Medications Among Patients With Bipolar Disorder, Psychiat. Servic., 58 (2007) 855-863.

10. H. Sadeghipour-Roudsari, M. Farahani, A. Shokrgozar, H. Farsam, A. Dehpour, Decrease in Erythrocyte: Plasma Lithium Ratio by Concurrent Administration of Psychotropic Drugs and Lithium in Mice, Gen. Pharmac., 31 (1998) 63–66.

11. T.W. McDade, S. Williams, J. Snodgrass, What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research, 4 (2007) 899-925.

12. M.V. Antunes, M.F. Charão, R. Linden, Dried blood spots analysis with mass spectrometry: Potentials and pitfalls in therapeutic drug monitoring, Clin. Biochem., 49 (2016) 1035–1046.

13. L. Baietto, A. D'Avolio, S. Pace, M. Simiele, C. Marra, A. Ariaudo, G. Di Perri, F. Rosa, Development and validation of an UPLC–PDA method to quantify daptomycin in human plasma and in dried plasma spots, J. Pharmac. Biomed. Anal., 88 (2014) 66–70.

14. N. Kostić, Y. Dotsikas, N. Jović, G. Stevanović, A. Malenović, M. Medenica, Quantitation of pregabalin in dried blood spots and dried plasma spots by validated LC–MS/MS methods, J. Pharmaceut. Biomed. Anal., 109 (2015) 1-28.

15. L. Rhoden, M.V. Antunes, P. Hidalgo, C.A. da Silva, R. Linden, Simple procedure for determination of valproic acid in dried bloodspots by gas chromatography–mass spectrometry, J. Pharm. Biom. Anal., 96 (2014) 207–212.

16. E.J.J. Berm, J. Paardekooper, E. Brummel-Mulder, E. Hak, B. Wilffert, J.G. Maring, A simple dried blood spot method for therapeutic drug monitoring of the tricyclic antidepressants amitriptyline, nortriptyline, imipramine, clomipramine, and their active metabolites using LC-MS/MS, Talanta 134 (2015) 165–172.

17. FDA, Guidance for Industry: Bioanalytical Method Validation, Guid. Ind. Bioanal. Method Valid. (2001) 4–10. doi:http://www.labcompliance.de/documents/FDA/FDA-Others/Laboratory/f-507-bioanalytical-4252fnl.pdf.

18. European Medicines Agency, Guideline on Bioanalytical Method Validation, European Medicines Agency (2011) 4–10.

19. R.E.S. Froes, C.C. Windmöller, J.B.B. da Silva, Emprego da modificação química permanente para a determinação de metais em matrizes diversas por espectrometria de absorção atômica em forno de grafite. Revista Analytica 23 (2006) 32-43.

20. P. Timmerman, S. White, S. Globig, S. Lüdtke, L. Brunet, J. Smeraglia, EBF recommendation on the validation of bioanalytical methods for dried blood spots, Bioanalysis. 14 (2011) 1567–1575.

21. M.C.G. Guilherme, Lithium carbonate: Atomic Absorption Spectrometry application in bioequivalence and profile use in pediatrics. 2007 Doctorate thesis. University of São Paulo, Brazil.

22. S. Capiau, V.V. Stove, W.E. Lambert, C.P. Stove, Prediction of the hematocrit of dried blood spots via potassium measurement on a routine clinical chemistry analyzer, Anal. Chem., 85 (2013) 404–410.

5. CONSIDERAÇÕES FINAIS

O Li é considerado o mais eficaz dentre todos os fármacos utilizados no tratamento do TAB, porém a faixa terapêutica estreita e a ocorrência de efeitos adversos contribuem para a baixa adesão ao tratamento.

O MTF tem como objetivo alcançar a eficácia terapêutica, através do ajuste de doses conforme as diferenças farmacocinéticas individuais, reduzindo o risco de toxicidade e aumentando a adesão ao tratamento do ponto de vista correto, com relação as doses e aos intervalos de administração. Para avaliar a relação entre dose e eficácia, o monitoramento das concentrações de Li no organismo é realizado usualmente em amostras de soro, as quais necessitam de cuidados especiais no transporte.

O uso de DBS surgiu como estratégia alternativa de amostragem, a qual revolucionou o monitoramento terapêutico de fármacos, pois além das coletas serem minimamente invasivas, podem ser realizadas pelo próprio paciente em papel filtro, que mantém a estabilidade dos analitos sem sofrer influência da temperatura, além de inativar os patógenos possivelmente presentes na amostra, permitindo o transporte através do serviço postal convencional.

Da mesma forma, o DPS também surgiu como uma estratégia alternativa de amostragem, porém oferece vantagens sobre o DBS, pois não sofre influência do hematócrito. Além disso, a faixa terapêutica do Li é estabelecida a partir das concentrações séricas, o que facilita o estabelecimento dos níveis terapêuticos também para o DPS. No entanto, estas estratégias de amostragem oferecem limitações, as quais estão relacionadas ao baixo volume disponível de amostra, necessitando de técnicas analíticas sensíveis para a determinação das concentrações de Li. Portanto, a técnica analítica de escolha para determinar os níveis de Li nessas amostras é a espectrofotometria de absorção atômica, devido a sua seletividade, sensibilidade e capacidade de análise com mínima preparação.

No presente estudo, as metodologias para a determinação de Li em amostras de DBS e DPS, empregando EAAFG foram satisfatórias considerando a necessidade de técnicas sensíveis, específicas e com preparação facilitada, permitindo detectar os analitos mesmo em concentrações baixas em pequenos volumes de amostras. Com um único disco de DBS de 8 mm foi possível fazer a análise, atendendo aos critérios de validação.

As limitações do DBS estão relacionadas à exatidão e ao rendimento da extração, que podem ser afetados pelo Hct nas análises. Portanto, foram avaliados os impactos do Hct na

exatidão e no rendimento da extração, nos Hct 25, 45 e 55%, empregando três níveis de controles (CQB, CQM e CQA). A exatidão ficou na faixa de 85-115%, sendo o menor valor observado na amostra controle com Hct no valor de 25%, devido a menor viscosidade do sangue, no que resulta em um volume menor de sangue no disco de 8 mm. Por outro lado o maior valor foi observado nas amostras controle com Hct de 55%, devido ao maior volume de sangue no disco de 8 mm. A exatidão pode ser corrigida se o volume de sangue aplicado no papel for fixo, utilizando toda a mancha na extração, ou com o conhecimento do Hct do paciente ao fazer-se a correção do resultado pelo volume obtido com as manchas de 8 mm nos diferentes valores de Hct (15, 18 e 20 µl para Hct de 25, 45 e 55%, respectivamente). Ao fazer-se esta estimativa, os valores de exatidão melhoram para 92 a 105%, sem diferenças proporcionais entre os diferentes Hct.

O rendimento da extração de lítio ficou na faixa de 83-93%, sendo calculado dividindo a percentagem média das absorbâncias das amostras controles pelos valores de absorbância das soluções controles. O rendimento da extração não foi afetado pelo Hct, porém foi moderadamente afetado pela concentração, sendo pouco maior em concentrações mais altas, entretanto este aspecto não afeta a exatidão do ensaio, visto que os calibradores abrangem intervalo amplo de concentrações.

O impacto do volume de sangue na exatidão também foi avaliado, testando-se diferentes volumes de sangue (30, 40 e 60 µL) para mimetizar as variações nos volumes das gotas de sangue capilar obtidas nas coletas de pacientes. Não houve impacto significativo na exatidão das concentrações de Li medidas em DBS, apresentando valores na faixa de 88-112%.

Da mesma forma, para o DPS também foi avaliado o impacto do volume de plasma na exatidão, testando-se diferentes volumes de plasma (15, 20 e 25 µL), para mimetizar as variações nos volumes de plasma obtidos nos dispositivos de coleta de DPS. Assim como nas amostras de DBS, os volumes não tiveram impacto na exatidão dos níveis de Li medidos nas amostras de DPS, apresentando valores entre 100 e 109%.

A medida sanguínea pode ser utilizada como uma medida complementar a sérica, pois permite estimar as concentrações eritrocitárias se ambas estiverem associadas, visto que o aumento dos níveis eritrocitários sobre os níveis plasmáticos está relacionado a ocorrência de toxicidade. Uma das vantagens oferecidas com relação ao uso de amostras secas é a maior estabilidade do lítio, dispensando a necessidade de refrigeração, pois se mantiveram estáveis por até 20 dias em diferentes condições de armazenamento. É importante ressaltar que ainda não há descrição na literatura de metodologia para análise de lítio em amostras de DBS e DPS.

Os pacientes, cujas amostras de DPS apresentaram irregularidades como volume reduzido da mancha de plasma ou extravasamento de sangue durante a passagem pela membrana de polissufonato nanoestruturado do dispositivo foram excluídos do estudo. As amostras de DPS seriam representativas dos níveis séricos. Os resultados deste estudo indicam que há uma alta correlação entre as concentrações de Li entre amostras de DPS e soro e as dosagens em DBS podem ser complementares ao monitoramento. Entretanto, não foi possível estimar satisfatoriamente os níveis séricos de Li a partir das medidas em DBS, visto que há diversos fatores que podem levar a variabilidade no transporte do Li para as hemácias. Entretanto, as amostras de DPS podem ser utilizadas juntamente com as concentrações em DBS para o cálculo do índice eritrocitário de Li.

A variabilidade nos níveis de Li e a alta frequência de pacientes com níveis séricos abaixo do intervalo terapêutico poderiam ser em virtude da baixa adesão ao tratamento. Até o momento apenas a dosagem sérica de Li tem sido utilizada para avaliar a adesão e a eficácia terapêutica. Porém, a dosagem de Li em DBS e DPS pode ser mais atrativa no monitoramento devido à sua praticidade na coleta e no transporte e podem ser consideradas em novos estudos. Sendo assim, as estratégias bioanalíticas desenvolvidas neste estudo podem ser utilizadas para a dosagem de Li com elevada sensibilidade e especificidade, em sangue total e em plasma além da dosagem sérica, obtendo-se resultados mais robustos na avaliação da eficácia terapêutica, permitindo a expansão do MTF.

REFERÊNCIAS

ANTUNES, Marina Venzon; CHARÃO, Mariele Feiffer; LINDEN, Rafael. **Dried blood spots analysis with mass spectrometry: Potentials and pitfalls in therapeutic drug monitoring.** Clinical Biochemistry. v. 49, p. 1035–1046, 2016.

BAIETTO, **Lorena et al. Development and validation of an UPLC–PDA method to quantify daptomycin in human plasma and in dried plasma spots.** Journal of Pharmaceutical and Biomedical Analysis. v. 88, p. 66–70, 2014.

BALKHI, Souleiman El; et al. **Lithium poisoning: Is determination of the red blood cell lithium concentration useful?** Clinical Toxicology. v. 47, p. 8–13, 2009.

BRASIL, MINISTÉRIO DA SAÚDE, 2016. **Protocolo clínico e diretrizes terapêuticas Transtorno afetivo bipolar do tipo I.** Secretária de Atenção à Saúde. Disponível em <http://conitec.gov.br/images/Protocolos/PCDT_TranstornoAfetivoBipolar_TipoI.pdf> Acesso em 28/03/2017

CAMUS, M. et al. **Comparison of lithium concentrations in red blood cells and plasma in samples collected for TDM, acute toxicity, or acute-on-chronic toxicity.** European Journal of Clinical Pharmacology. v. 59, p. 583-587, 2003.

CARSON SW. et al. **Principles of therapeutic drug monitoring**. 3.ed. Applied Pharmacokinetics, Vancouver, 1992.

CUPERTINO, Leidiane Heleno et al. **Medicamentos anti-hipertensivos dispensados em uma unidade básica de saúde no município de ipatinga-MG**. Única cadernos acadêmicos, v. 3, n. 1, p. 1-13, 2016.

DE LIMA, Gabriela Martins Silva et al. **Determinação simultânea de carbamazepina, fenitoína e fenobarbital em sangue seco em papel por cromatografia líquida de alta eficiência.** Química Nova. v. 37, p. 1067-1071, 2014.

DE OLIVEIRA, Jobson Lopes de et al. **Lithium nephrotoxicity**. Revista da Associação Médica Brasileira, v. 56, n. 5, p. 600-606, 2010.

DE SOUZA, Camila et al. **Transtorno bipolar e medicamentos: adesão, conhecimento dos pacientes e monitorização sérica do carbonato de lítio**. Revista Latino-Americana de Enfermagem, v. 21, n. 2, p. 624-631, 2013.

DOS SANTOS, Lisia Maria G dos; GONÇALVES Jaylei M.; JACOB, Silvana do Couto. **Determinação simultânea de As, Cd e Pb em amostras de água purificada para hemodiálise por espectrometria de absorção atômica com forno de grafite, após otimização multivariada baseada no uso de planejamento experimental** Química Nova. v. 31, p. 975-979, 2008.

DUQUE, Manuel Enrique Machado et al. **Perfil de utilización del carbonato de litio en pacientes com trastorno afectivo bipolar en 25 ciudades de Colombia** Biomédica. v. 37, p. 43-50, 2017.

FELLINGER, Matthaus et al. **Influence of gender on inpatient treatment for bipolar disorder: An analysis of 60,607 hospitalisations** Journal of Affective Disorders. v. 225, p. 104–107, 2018.

FROES, Roberta Eliane Santos; WINDMÖLLER, Cláudia Carvalhinho; da SILVA, José Bento Borba. **Emprego da Modificação Química Permanente para a determinação de metais em matrizes diversas por Espectrometria de Absorção Atômica em Forno de Grafite** Revista Analytica. v. 23, p. 32-43, 2006.

GOODMAN, L.L.; GILMAN, B. **As bases farmacológicas da terapêutica.** 11ª Edição. Rio de Janeiro: Guanabara Koogan, p. 433-439, 2006.

HANEMANN, Fernanda Della Méa. **Carbonato de lítio.** Revista da Graduação, v. 3, p. 1-37, 2010.

HIEMKE, C. et al. **AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011**. Pharmacopsychiatry, v. 21, n. 06, p. 195-235, 2011.

KIM, Hee Jung; THAYER, Stanley A. **Lithium increases synapse formation between hippocampal neurons by depleting phosphoinositides.** Molecular pharmacology, v. 75, n. 5, p. 1021-1030, 2009.

KOSTIC, Nada et al. **Quantitation of pregabalin in dried blood spots and dried plasma spots by validated LC–MS/MS methods** Journal of Pharmaceutical and Biomedical Analysis. v. 109, p. 1-28, 2015.

KUMMER, Carmen Luize; COELHO, T. C. R. B. **Antiinflamatórios não esteróides inibidores da ciclooxigenase-2 (COX-2): aspectos atuais.** Rev Bras Anestesiol, v. 52, n. 4, p. 498-512, 2002.

LEAL, A. C. M., AND A. S. G. FERNANDES. **Lítio e a sua aplicação terapêutica na psicose maníacodepressiva.** Biologias-Revista de divulgação científica da ordem dos biólogos.[Citado em 3 de março de 2015]. Disponível em:< http://www. ordembiologos. pt/Publicacoes/Biologias/4_Litio (2002).

LIN, Yen-Feng; HUANG, Ming-Chyi; LIU, Hsing-Cheng. **Glycogen synthase kinase 3β gene polymorphisms may be associated with bipolar I disorder and the therapeutic response to lithium**. Journal of affective disorders, v. 147, n. 1-3, p. 401-406, 2013.

MARCOLIN, Marco Antonio; DA GRAÇA CANTARELLI, Maria; JUNIOR, Manoel Garcia. **Interações farmacológicas entre medicações clínicas e psiquiátricas.** Archives of Clinical Psychiatry, v. 31, n. 2, p. 70-81, 2004.

NASCIMENTO, Nara F. et al. **Alcohol and lithium have opposing effects on the period and phase of the behavioral free-running activity rhythm**. Alcohol, v. 49, n. 4, p. 367-376, 2015.

NIERENBERG, Andrew A. et al. **Lithium treatment moderate-dose use study (LiTMUS) for bipolar disorder: a randomized comparative effectiveness trial of optimized personalized treatment with and without lithium.** American Journal of Psychiatry, v. 170, n. 1, p. 102-110, 2013.

ORUCH, Ramadhan et al. **Lithium: A review of pharmacology, clinical uses, and toxicity** European Journal of Pharmacology v. 740, p. 464–473, 2014.

PARKER, Suzanne L. et al. **A validated method for the quantification of fosfomycin on dried plasma spots by HPLC-MS/MS: application to a pilot pharmacokinetic study in humans** Journal of Pharmaceutical and Biomedical Analysis. v. 115, p. 509-514, 2015.

PATON, Carol et al. **Lithium in bipolar and other affective disorders: prescribing practice in the UK**. Journal of Psychopharmacology, v. 24, n. 12, p. 1739-1746, 2010.

ROSA, Adriane Ribeiro et al. **Monitoramento da adesão ao tratamento com lítio** Revista de Psiquiatria Clínica. v. 33, p. 249-261, 2006.

SEVERUS, W. E. et al. **In search of optimal lithium levels and lanzapine doses in the long-term treatment of bipolar I disorder. A post-hoc analysis of the maintenance study by Tohen et al. 2005** European Psychiatry v. 25, p. 443–449, 2010.

SHAH, V.P. et al. **Analytical methods validation: Bioavailability, bioequivalence and pharmacokinetic studies** European Lournal of Drug Metabousm and Pharmacokinetics. v. 16, p. 249-255, 1991.

ANEXO I

Instrução para autores da revista Talanta para a submissão do artigo intitulado *"Determination of lithium in Dried Blood Spots and Dried Plasma Spots by Graphite Furnace Atomic Absorption Spectrometry: Method development, validation and clinical application"*

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19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
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Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
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[5] Cancer Research UK, Cancer statistics reports for the UK.
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Você está sendo convidado a participar do projeto institucional: **Manchas de sangue seco em papel (DBS), plasma seco em papel (DPS) e saliva como estratégias de amostragem alternativas no monitoramento terapêutico do lítio em pacientes com transtorno bipolar.** O objetivo deste estudo é estabelecer novas estratégias para o monitoramento terapêutico do lítio em pacientes com transtorno afetivo bipolar, empregando amostras de sangue seco em papel, plasma seco em papel e saliva. O estudo busca também caracterizar um grupo de pacientes da cidade de Novo Hamburgo com transtorno bipolar em tratamento com carbonato de lítio, considerando aspectos sóciodemográficos, conhecimentos e atitudes com relação à medicação e o perfil de adesão.

Sua participação nesta pesquisa será voluntária e consistirá em fornecer dados sociodemográficos como idade, peso, altura, estado civil, escolaridade renda familiar; dados socioculturais como hábito de fumo, consumo de bebida alcóolica, prática de exercício físico; e informações relacionados com o tratamento com o lítio, como tempo em tratamento, presença de efeitos adverso, uso de outras medicações. Você também será solicitado a responder questionários que indicam o nível de adesão a medicação. Como o estudo objetiva avaliar monitorar os níveis da medicação, iremos coletar amostras de sangue e saliva a cada seis meses, totalizando 3 coletas. Para tal, serão coletadas duas gotas de sangue capilar dos dedos médio ou indicador com lanceta apropriada e colocada em papel filtro. Posteriormente, através da punção venosa será colhida amostra de 4 mL de sangue venoso do seu braço. Também será realizada a coleta de amostra de saliva, em que você colocará um rolo de algodão na boca e após ficar embebido de saliva, cerca de dois minutos, será armazenado em tubo plástico. Durante a realização do estudo as amostras permanecerão armazenadas a -20 °C no laboratório em que serão executadas as análises.

Os possíveis riscos da participação no estudo envolvem as coletas de sangue venoso e capilar que podem gerar desconforto e dor em virtude da punção venosa e transcutânea,

UNIVERSIDADE

Nome do pesquisador responsável: Marina Venzon Antunes

Telefone institucional do pesquisador responsável: 3586.8800 (ramal 9039)

E-mail institucional do pesquisador responsável: marinaantunes@feevale.br

_____

Assinatura do pesquisador responsável

Local e data: _____, _____ de _____20____.

Declaro que li o TCLE: concordo com o que me foi exposto e aceito participar da pesquisa proposta.

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Manchas de sangue seco em papel (DBS), plasma seco em papel (DPS) e saliva como estratégias de amostragem alternativas no monitoramento terapêutico do lítio em pacientes com transtorno bipolar

Pesquisador: Marina Venzon Antunes

Área Temática:

Versão: 2

CAAE: 69910317.1.0000.5348

Instituição Proponente: ASSOCIACAO PRO ENSINO SUPERIOR EM NOVO HAMBURGO

Patrocinador Principal: ASSOCIACAO PRO ENSINO SUPERIOR EM NOVO HAMBURGO

DADOS DO PARECER

Número do Parecer: 2.231.794

Apresentação do Projeto:

De acordo.

Objetivo da Pesquisa:

De acordo.

Avaliação dos Riscos e Benefícios:

De acordo.

Comentários e Considerações sobre a Pesquisa:

De acordo.

Considerações sobre os Termos de apresentação obrigatória:

De acordo.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

De acordo.

UNIVERSIDADE FEEVALE

UNIVERSIDADE FEEVALE COMITÊ DE ÉTICA EM PESQUISA - CEP

UNIVERSIDADE FEEVALE/ASSOCIAÇÃO PRÓ-ENSINO SUPERIOR EM NOVO

Plataforma Brasil

Continuação do Parecer: 2.231.794

Considerações Finais a critério do CEP:

Em conformidade com a Resolução nº 466 de 12 de dezembro de 2012, do Conselho Nacional de Saúde, e com as normas internas do Comitê de Ética em Pesquisa da Universidade Feevale, todos os documentos necessários à análise do projeto acima referido por este Comitê foram apresentados.

Este projeto preserva os aspectos éticos dos sujeitos da pesquisa, sendo, portanto, aprovado pelo Comitê de Ética em Pesquisa da Universidade Feevale.

Reiteramos que o Comitê de Ética em Pesquisa da Instituição encontra-se à sua disposição para equacionar eventuais dúvidas e/ou esclarecimentos que se fizerem necessários.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_939283.pdf	14/08/2017 21:38:06		Aceito
Outros	carta_pendencias_parecer_CEP.docx	14/08/2017 21:37:25	Marina Venzon Antunes	Aceito
Outros	formulario_encaminhamento_CEP.pdf	14/08/2017 21:36:53	Marina Venzon Antunes	Aceito
Outros	termo_autorizacao_numesc.pdf	14/08/2017 21:35:25	Marina Venzon Antunes	Aceito
Declaração de Instituição e Infraestrutura	declaracao_instituicao_coparticipante.pdf	14/08/2017 21:34:28	Marina Venzon Antunes	Aceito
Declaração de Pesquisadores	declaracao_compromisso_pesquisador_respons.pdf	14/08/2017 21:33:53	Marina Venzon Antunes	Aceito
Folha de Rosto	folha_rosto_assi.pdf	14/08/2017 21:32:32	Marina Venzon Antunes	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	19/06/2017 10:14:41	Marina Venzon Antunes	Aceito
Outros	fichas_Avaliacao_questionarios.pdf	17/06/2017 17:37:29	Marina Venzon Antunes	Aceito
Projeto Detalhado / Brochura Investigador	projeto_litio_plataforma.docx	17/06/2017 17:35:17	Marina Venzon Antunes	Aceito

Situação do Parecer:
Aprovado