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Desenvolvimento e validação de método por LC-MS/MS para determinação de vancomicina em amostras de plasma em papel filtro seco

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Dissertação submetida ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre.

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“Querem que vos ensine o modo de chegar à ciência verdadeira?
Aquilo que se sabe, saber que se sabe;
aquilo que não se sabe, saber que não se sabe;
na verdade,é este o saber.”

Confúcio

“Na vida nada deve ser temido, apenas compreendido”.

Marie Curie

RESUMO

A vancomicina é considerada um agente antimicrobiano eficaz para o tratamento de infecções gram-positivas graves. A importância do monitoramento terapêutico dos antimicrobianos tem levado ao desenvolvimento de técnicas analíticas mais específicas, capazes de identificar com precisão a concentração dessa substância no organismo. Assim, nesse trabalho foi proposta uma metodologia para determinação de vancomicina em amostras de plasma em papel filtro seco e análise por cromatografia líquida acoplada à espectrometria de massas. Uma alíquota de 10 µL de plasma foi transferida para o disco de papel e completamente seca em temperatura ambiente. A extração foi realizada após papel ter sido cortado e transferindo para um micro tubo de polipropileno, utilizando-se tampão fosfato de sódio, e padrão interno polimixina. A mistura foi agitada, centrifugada e uma alíquota de 5 µL foi injetada no sistema UHPLC-MS / MS. A otimização foi conduzida por planejamento experimental e validada; os valores de precisão intra e inter-dia, nos níveis de concentração mais baixos, foram sempre inferiores a 20%, considerando seu desvio padrão relativo. Quanto aos valores de exatidão, estes também foram satisfatórios (acima de 80%). Este método foi aplicado com sucesso a 75 amostras de pacientes submetidos à terapia com vancomicina. Este trabalho conseguiu atingir seus objetivos de otimizar, validar a metodologia analítica e analisar amostras de plasma de pacientes em uso de vancomicina. O método apresentou boa correlação com o método imunoenzimático atual, sendo possível sua aplicabilidade na prática clínica de acompanhamento terapêutico.

Palavras Chave: Manchas de plasma seco, vancomicina, monitoramento terapêutico de fármacos, toxicologia clínica, LC-MS / MS.

ABSTRACT

Vancomycin is used as antimicrobial agent for the treatment of severe gram-positive infections. The importance of therapeutic monitoring of antimicrobials has led to the development of more specific analytical techniques capable of identifying with accuracy the concentration of this substance in the organism. Thus, in this work, a methodology for the determination of vancomycin in plasma samples on dry filter paper and analysis by liquid chromatography coupled to mass spectrometry was proposed. An aliquot of 10 µL of plasma was transferred on the paper disk and completely dried under at room temperature. The extraction was performed after the paper had been cut and transferred to a to a polypropylene microtube, using sodium phosphate buffer, and internal standard polymyxin. The mixture was shaken, centrifuge, and a 5 µL aliquot was injected into the UHPLC-MS/MS system. The optimization was conducted by experimental planning and validated; the intra and inter-day precision values, at the lowest concentration levels, were always less than 20%, considering their relative standard deviation. As for the accuracy values, these were also satisfactory (above 80%). This method was successfully applied to 75 samples of patients undergoing vancomycin therapy. This work was able to achieve its goals of optimizing, validating the analytical methodology and analyzing plasma samples from patients using vancomycin. The method showed good correlation with the current immunoenzymatic method, and its applicability in the clinical practice of therapeutic monitoring is possible.

Keywords: Dried plasma spots, vancomycin, therapeutic drug monitoring, clinical toxicology-MS/MS.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

Agr	Gene regulador acessório, do inglês: *accessory gene regulatory*
DPS	Plasma seco em papel filtro, do inglês: *dried plasm spot*
DS	Amostra em papel seco, do inglês: *dried spot*
GC	Cromatografia gasosa, do inglês: *gas chromatography*
EMIT	Imunoensaios enzimático de multiplicação, do inglês: *enzyme multiplied immunoassay technique*
ESI	Ionização por *electrospray*
FIA	Imunoensaio de fluorescência, do inglês: *fluorescence immunoassay*
FPIA	Imunofluorescência polarizada, do inglês: *fluorescence polarization immunoassay*
HPLC	Cromatografia líquida de alta eficiência, do inglês: *high performance liquid chromatography*
LC-MS/MS	Cromatografia líquida acoplada espectrometria de massas sequencial, do inglês: *liquid chromatography – mass spectrometry (tandem)*
HPS	Hospital de Pronto Socorro
IV	Via intravenosa
LC-MS	Cromatografia líquida acoplada a espectrometria de massa, do inglês: *Liquid chromatography–mass spectrometry*
MRSA	*Staphylococcus aureus* resistente à meticilina, do inglês: *Methicillin-resistant Staphylococcus aureus*
MS	Espectrometria de massa, do inglês: *Mass Spectrometry*
PBP	Proteína ligadora de penicilina, do inglês: *Penicillin-binding protein*
RIA	Radioimunoensaio, do inglês: *Radioimmunoassay*
SCCmec	Cassete cromossômico estafilocócico mec. do inglês: *Staphyococcal chromosome cassette mec*
TDM	Monitoramento terapêutico de fármacos, do inglês: *Therapeutic Drug Monitoring*
UHPLC-MS/MS	Cromatografia líquida de ultra alto desempenho acoplada a espectrometria de massas sequencial, do inglês: *Ultra-high performace liquid chromatography - mass spectrometry (tandem)*
UTI	Unidade de Terapia Intensiva

VISA	*Staphylococcus aureus* resistência intermediária à vancomicina, do inglês: *vancomycin intermediate-resistant Staphylococcus aureus*
VRSA	*Staphylococcus aureus* resistente à vancomicina, do inglês: *vancomycin-resistant Staphylococcus aureus*

SUMÁRIO

1. INTRODUÇÃO

A vancomicina é um antibiótico glicopeptídeo amplamente utilizado no tratamento de infecções graves por bactérias gram-positivas, incluindo infecções por *Staphylococcus aureus* resistente à meticilina (MRSA) (ELBARBRY, 2017; JAVORSKA *et al.*, 2016; OKANO; ISLEY; BOGER, 2017) que apresentam elevada ocorrência em unidades de terapia intensiva nos hospitais (DELWING, 2015; DOMBROSKI; SILVA; SILVEIRA, 2015). O uso excessivo ou inadequado de antimicrobianos é responsável, pelo surgimento de microrganismos multirresistentes, causando um aumento na morbimortalidade dos pacientes e, consequentemente, impactando em custos adicionais aos sistemas de saúde (DELWING, 2015). De acordo com a Organização Mundial de Saúde, a resistência a antimicrobianos é um problema de saúde mundial há décadas, responsável por, aproximadamente, 700 mil mortes anuais, sendo o *Staphylococcus aureus* resistente à meticilina, enquadrado como patógeno prioritário neste cenário mundial (WHO, 2017).

O aumento nas taxas de incidência, a mudança no padrão epidemiológico das cepas MRSA, associado à dificuldade de tratamento das infecções causadas pelo *Staphylococcus aureus*, têm sido um desafio a saúde pública mundial (MONTEIRO SALES; DA SILVA, 2012). O aumento da prevalência de infecções hospitalares e comunitárias provocadas por MRSA e por outros microrganismos que são resistentes à penicilina, tem causado um aumento do uso da vancomicina (LUNA; RODRIGUEZ-; GOTUZZO, 2010; RYBAK *et al.*, 2009). Neste cenário, o monitoramento terapêutico é altamente recomendado devido ao seu estreito índice terapêutico e sua toxicidade (JAVORSKA *et al.*, 2016) visando garantir níveis adequados, eficazes e seguros (BARCO *et al.*, 2016).

Os ensaios para monitoramento de fármacos são geralmente realizados em soro ou plasma obtido por punção venosa. Entretanto, há um crescente interesse no uso de novas técnicas de bioamostragem, que incluem amostras de plasma seco em papel filtro (DPS), em virtude de vantagens como: pequeno volume; uma maior estabilidade da maioria dos analitos, facilidade no armazenamento e transporte (PARKER *et al.*, 2016; WAGNER *et al.*, 2016; WILHELM; DEN BURGER; SWART, 2014). Adicionalmente, os métodos laboratoriais de rotina para determinação e quantificação da vancomicina incluem os imunoensaios pela rapidez, simplicidade de execução e disponibilidade de instrumentação analítica, no entanto estes têm demonstrado ter uma falta de especificidade promovida pela reatividade cruzada dos anticorpos ou a presença de substâncias interferentes (BARCO *et al.*, 2016; JAVORSKA *et al.*, 2016). Assim, sugere-se cromatografia líquida acoplada a espectrometria de massas, como

metodologia padrão ouro para a avaliação quantitativa do fármaco, pela sua elevada sensibilidade, conseguindo detectar baixas concentrações de fármacos em fluídos biológicos e especificidade necessária para aplicação clínica (BARCO *et al.*, 2016).

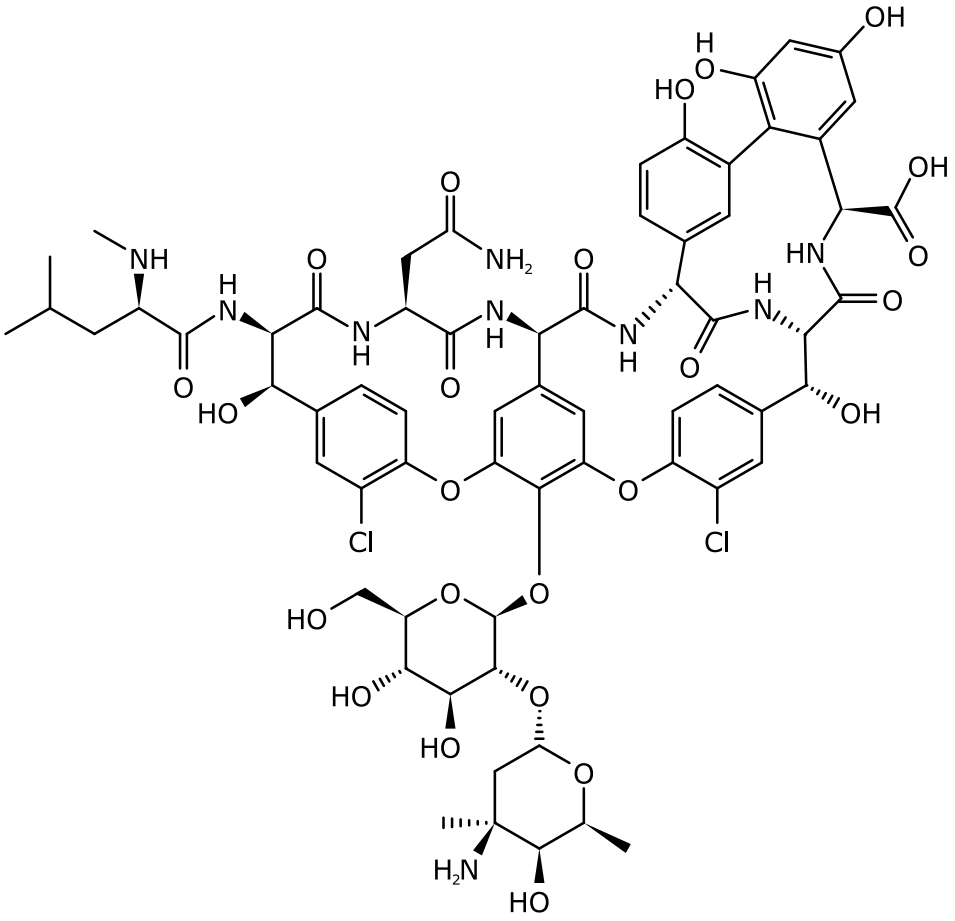
Diante do exposto, constata-se a importância da monitorização terapêutica de antimicrobianos como a vancomicina na prática clínica, a qual tem levado ao desenvolvimento de técnicas analíticas que sejam mais específicas e capazes de identificar com maior rapidez e exatidão a concentração desta substância no organismo. Além disso, preconizam-se métodos que utilizem o menor volume de solventes orgânicos, que sejam rápidos, fáceis de serem executados, que exijam um baixo custo operacional e que tenha aplicabilidade na prática clínica de monitoramento terapêutico.

Neste contexto, o uso de microamostras secas, como amostras de plasma seco em papel filtro, por possibilitar uma maior estabilidade da maioria dos medicamentos quando comparado aos espécimes líquidos, permite o armazenamento e transporte facilitado, através de serviços de correios não refrigerados. Considerando o uso potencial de DPS para aumentar o acesso ao monitoramento terapêutico de vancomicina, em locais de recursos limitados que não possuem laboratórios com tecnologia e equipamentos analíticos adequados, este estudo apresenta uma importante aplicabilidade social por permitir a redução dos custos de transporte e armazenamento de espécimes viáveis a análise, em locais com recursos limitados e aumento do acesso ao monitoramento terapêutico, visando a melhoria da prática clínica e redução dos gastos hospitalares, através do desenvolvimento, padronização e validação de um método analítico utilizando a cromatografia líquida acoplada a espectrometria de massas em tandem (LC-MS/MS) para determinação de vancomicina em amostras de DPS.

2. REFERENCIAL TEÓRICO

A vancomicina é um antibiótico glicopeptídeo tricíclico complexo, produzido pelo *Streptomyces orientalis* (BRUNTON; BJORN, 2012), isolado em 1956 e introduzido na prática clínica, através da aprovação pelo *Food and Drug Administration* (FDA) nos Estados Unidos da América em 1958 (DELWING, 2015). Apresenta a fórmula molecular $C_{66}H_{75}Cl_2N_9O_{24}$, peso molecular 1449,3 g/mol, é um composto polar altamente solúvel em água, moderadamente solúvel em metanol, insolúvel em álcoois superiores, acetona e éter. Em solução a 5% de água tem um pH 2.5 a 4.5 (SWEETMAN, 2009). A estrutura química da vancomicina é apresentada na **Figura 1**.

Figura 1. Estrutura química da vancomicina.



Por apresentar baixa absorção após a administração oral, a vancomicina na maioria das vezes é administrada por via intravenosa (IV). Uma dose IV de 1 g, em adultos com função renal normal, gera uma concentração plasmática de 15-30 μg/mL, após 1 hora de uma infusão com 1-2 horas de duração, tem uma meia vida de eliminação sérica de cerca de 6 horas e cerca de 30% do fármaco liga-se às proteínas plasmáticas. Cerca de 90% de uma dose injetada é excretada via filtração glomerular, portanto, um comprometimento da função renal pode fazer com que ocorra um acumulo de fármaco no organismo causando toxicidade (BRUNTON; BJORN, 2012). É indicada no tratamento de infecções graves causadas por bactérias gram-positivas como MRSA (DOMBROSKI; SILVA; SILVEIRA, 2015), mas também em casos de pneumonia, empiema, endocardite, osteomielite e abscessos dos tecidos moles e no tratamento de infecções estafilocócicas graves em pacientes alérgicos às penicilinas e às cefalosporinas (BRUNIERA *et al.*, 2015; BRUNTON; BJORN, 2012). Este agente antimicrobiano é amplamente utilizado em hospitais, sendo o fármaco de primeira escolha para o tratamento infecções por cepas de *Staphyloccus aureus* produtoras de penicilinase (RYBAK *et al.*, 2009) e de sepse de pacientes internados em unidade de terapia intensiva (UTI) (DELWING, 2015) para tratamento de infecções causadas por bactérias gram-positivas como estafilococos, enterococos e pneumococos (TAVARES, 2007).

Os principais efeitos adversos da vancomicina são a nefrotoxicidade (ELBARBRY, 2017) e ototoxicidade (OLSON, 2014), podendo levar a insuficiência renal e a surdez permanente, associado a altas concentrações (TAVARES, 2007). Entre as reações de hipersensibilidade à vancomicina destacam-se as erupções cutâneas maculares e a ocorrência de anafilaxia. A infusão IV rápida pode causar reações eritematosas ou urticariformes, rubor, taquicardia e hipotensão. O extremo rubor que pode ocorrer é às vezes denominado síndrome "do pescoço vermelho" ou "do homem vermelho" (BRUNIERA *et al.*, 2015; BRUNTON; BJORN, 2012; OLSON, 2014)

É considerada um medicamento com propriedades bactericidas para os microrganismos em divisão, pois inibe a síntese da parede celular das bactérias susceptíveis, através de sua ligação de alta afinidade à extremidade terminal D-alanil-D-alanina do peptídeoglicano da parede celular. Em consequência, o peptídeoglicano é enfraquecido, e a célula torna-se suscetível a lise. A membrana celular também é danificada, contribuindo para o efeito antimicrobiano (BRUNTON; BJORN, 2012).

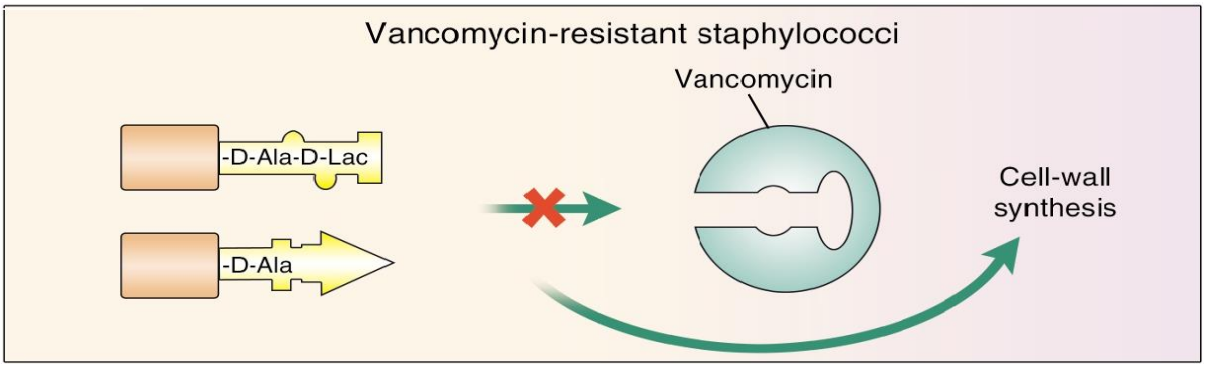
O *Staphylococcus aureus* pode adquirir resistência aos antimicrobianos por mutações em seus genes e/ou pela aquisição de genes de resistência de outras bactérias da mesma ou de outras espécies (CUSSOLIM et al, 2021). Os mecanismos pelos quais o *Staphylococcus aureus*

pode desenvolver resistência são: bomba de efluxo, inativação de aminoglicosídeos e modificação nas proteínas ligadoras de penicilinas (PBPs). O *Staphylococcus aureus* resistentes à meticilina (MRSA) são cepas resistentes a todos os antimicrobianos β-lactâmicos, seu mecanismo de resistência está relacionado ao desenvolvimento de uma proteína ligadora de penicilina (PBP) adicional, a PBP2a, que é plenamente funcional, mas não tem afinidade por antimicrobianos beta-lactâmicos (GELATTI; BECKER, 2009; SALES; SILVA, 2012). A codificação dessa nova PBP, torna esses patógenos resistentes à meticilina e está relacionada à aquisição do gene mecA. Esse gene é parte integrante de um elemento genômico denominado "cassete cromossômico estafilocócicas mecA" (SCCmec) (GELATTI; BECKER, 2009).

A resistência do *Staphylococcus aureus* à vancomicina, pode se expressar através de dois fenótipos distintos VISA (*Staphylococcus aureus* com sensibilidade reduzida à vancomicina) e VRSA (*Staphylococcus aureus* resistente à vancomicina) (BERTOLUCI, 2007). Os genes (van A) do enterococos resistente à vancomicina tem sido associada com um polimorfismo no gene regulador acessório (agr) (LUNA; RODRIGUEZ; GOTUZZO, 2010).

O desenvolvimento da resistência medicamentosa à vancomicina decorre da expressão de uma enzima singular que modifica o precursor da parede celular causando uma alteração do peptídeo terminal para D-Ala-D-Lac em vez de D-Ala-D-Ala (BRUNTON; BJORN, 2012). O mecanismo de resistência da vancomicina é apresentado na **Figura 2**. Os principais fatores de risco de resistência à vancomicina são a exposição anterior à vancomicina nos 30 dias que antecedem a coleta de cultura de MRSA e a permanência em uma unidade de terapia intensiva (UTI) onde a vancomicina é usada (LUNA; RODRIGUEZ; GOTUZZO, 2010)

Figura 2. Mecanismo de resistência do *Staphylococcus aureus* à vancomicina



Fonte: LOWY, p.1270,2003

Nesse contexto, o monitoramento terapêutico é considerado uma ferramenta para auxiliar no acompanhamento clínico, ajustando individualmente a dose de um fármaco baseada

na concentração medida no fluído biológico. Há vários critérios a serem considerados para ser capaz de executar de forma racional o monitoramento terapêutico de fármacos (TDM) como: uma boa relação entre a concentração do fármaco e a resposta farmacológica; um intervalo de concentração alvo definido; disponibilidade de um ensaio bioanalítico preciso, seletivo e rápido; uma grande variabilidade inter-individual na farmacocinética (JAGER *et al.*, 2016). Em um ambiente clínico, o TDM promove a individualização posológica do paciente, no sentido de equilibrar a eficácia terapêutica com o mínimo de efeitos indesejados do fármaco (ADAWAY; KEEVIL, 2012; KLAASSEN; WATKINS III, 2012; MOREAU; SIQUEIRA, 2016; OGA; CAMARGO; BATISTUZZO, 2014).

O uso da vancomicina ainda é muito comum nos hospitais, no entanto, doses inadequadas e terapia prolongada pode resultar em um aumento da ocorrência de cepas resistentes ou falhas de tratamento e também efeitos tóxicos, associado a elevadas concentrações (BRUNIERA *et al.*, 2015; KÖNIG *et al.*, 2013). Atualmente, é fundamental otimizar o tratamento das infecções de pacientes que fazem uso da vancomicina, considerando os princípios da farmacocinética e farmacodinâmica (DOMBROSKI; SILVA; SILVEIRA, 2015), para minimização da toxicidade, aumento da efetividade, melhoria do desfecho do tratamento e redução de cepas resistentes (OGA; CAMARGO; BATISTUZZO, 2014). A vancomicina é um antibiótico hidrofílico principalmente eliminado via filtração glomerular pelo rim, portanto um paciente com função renal insuficiente pode levar a uma longa meia-vida de eliminação e um nível sérico elevado do fármaco, aumentando o risco de toxicidade (ROBLES-PIEDRAS; GONZÁLEZ-LÓPEZ, 2009). Este fármaco também possui uma estreita faixa terapêutica e sua absorção apresenta grande variação entre os pacientes, pois depende da função renal, peso, idade e parâmetros metabólicos, portanto, a fim de obter a dose ideal, recomenda-se, além da adequação da dose empírica, o monitoramento da concentração sérica do fármaco e o subsequente ajuste da dose, caso necessário (BOAS, 2016).

O momento da coleta sanguínea é importante no monitoramento terapêutico, para a interpretação dos resultados. Para a monitorização terapêutica da vancomicina é recomendado que a coleta sanguínea seja realizada no vale (período de concentração mais baixa do medicamento), no terceiro dia de farmacoterapia, 30 minutos antes da quarta ou quinta dose para pacientes com função renal normal, momento em que é atingido o "*steady state*", ou seja, o estado de farmacocinética da vancomicina no organismo do paciente (ALMEIDA, 2011; DOMBROSKI; SILVA; SILVEIRA, 2015).

Mesmo que a vancomicina tenha sido utilizada clinicamente por mais de 60 anos (ELBARBRY, 2017), ainda existe controversas quanto a faixa terapêutica para este fármaco

(BRUNTON; BJORN, 2012; DOMBROSKI; SILVA; SILVEIRA, 2015; ELBARBRY, 2017). A posologia ideal para vancomicina é aquela que resulta em uma concentração plasmática com pico entre 40 e 50 mg/L e concentrações de vale entre 5 e 15 mg/L, recomendando-se que seja mantida uma concentração acima de 5 mg/L , para assegurar a sua eficácia e o valor máximo deve permanecer abaixo de 50 mg/L, para evitar a ototoxicidade (BERTOLUCI, 2007). As orientações clínicas atuais recomendam a utilização de doses elevadas de vancomicina, com manutenção de concentrações plasmáticas mínimas entre 15 a 20 mg/L (DOMBROSKI; SILVA; SILVEIRA, 2015), contudo, está descrito um aumento do risco de nefrotoxicidade nestas condições (SILVA, 2014).

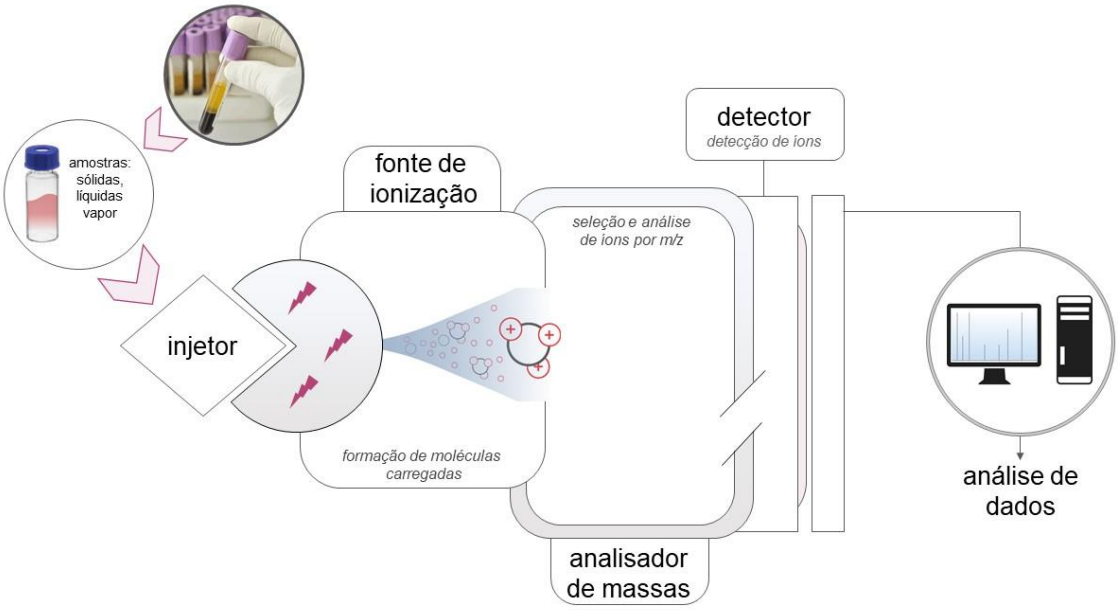
Diversos métodos analíticos para determinação e quantificação da vancomicina foram desenvolvidos e validados, incluindo métodos microbiológicos de difusão em ágar e turbidimétricos; métodos espectrométricos; métodos imunológicos como radioimunoensaio (RIA), imunoensaios de fluorescência (FIA), imunofluorescência polarizada (FPIA), imunoensaios enzimático de multiplicação (EMIT), cromatografia gasosa (GC), cromatografia líquida de alta eficiência (HPLC) e cromatografia líquida acoplada a espectrometria de massas (LC-MS) (BOTELHO, 2010).

Os imunoensaios continuam sendo os testes mais utilizados, pela rapidez, simplicidade de execução e pela disponibilidade de instrumentação analítica (BARCO *et al.*, 2016; BIJLEVELD *et al.*, 2014; OYAERT *et al.*, 2015; SILVA, 2014). Esses métodos são eficazes dentro da faixa de concentração terapêutica (5-20 μg/ml) com limite de quantificação para o EMIT de 5 μg/ml (USMAN; HEMPEL, 2016). Os imunoensaios utilizados na prática clínica sofrem algumas limitações, como: uma possível falta de seletividade e baixa especificidade, devido a reatividade cruzada dos anticorpos ou a presença de substâncias interferentes; há o inconveniente da dependência do fornecimento de consumíveis por empresas que detêm direitos comerciais e eleva o custo dos reagentes por amostra; o longo tempo necessário para o desenvolvimento de um novo ensaio (vinculado à produção de novos anticorpos monoclonais) (BOAS, 2016; WAGNER *et al.*, 2016). Os métodos cromatográficos são referência no monitoramento de fármacos e a técnica por LC-MS/MS é reconhecida como uma metodologia padrão ouro no monitoramento de fármacos pela elevada sensibilidade e especificidade, principalmente quando baixos níveis de vancomicina são esperados. Portanto, tem a vantagem de maior precisão e menor custo, uma vez que tenha sido feito o investimento inicial do equipamento (SILVA, 2014).

A espectrometria de massas (MS) é, nos dias de hoje, uma das técnicas analíticas mais versáteis em todas as áreas da ciência, em particular as de medicina, nanotecnologia e

biotecnologia, pela sua capacidade em detectar, quantificar e caracterizar átomos e moléculas dos mais variados tipos, composições e tamanhos. A técnica de MS é utilizada para analisar açúcares, peptídeos, proteínas, lipídeos, polímeros, nucleotídeos, drogas e metabólitos. Suas principais vantagens são a alta sensibilidade, seletividade e velocidade (LACERDA JR, 2018) e adequada para ser utilizada em análises toxicológicas, pois tem como grande vantagem sua elevada seletividade (ANDRADE FILHO; CAMPOLINA; DIAS, 2013). O princípio físico básico desta metodologia consiste em criar íons de compostos orgânicos, que são separados de acordo com a sua taxa de massa/carga (m/z), quando atravessam um campo eletromagnético (IGLESIAS, 2016; LACERDA JR, 2018; LANÇAS, 2013). O fluxograma do princípio físico da espectrometria de massas é apresentado na **Figura 3**.

Figura 3. Fluxograma do princípio físico da espectrometria de massas



Os ensaios para monitoramento de fármacos são geralmente realizados em diferentes matrizes biológicas com um crescente interesse no uso de novas técnicas de bioamostragem e microamostragem que incluem o DPS. Essa técnica consiste na deposição de pequenos volumes de plasma em cartões de papel filtro. Estes cartões geralmente, são de baixo custo, fáceis de fabricar, facilitam a identificação para a rastreabilidade da amostra e apresentam boas propriedades de adsorção (WAGNER *et al.*, 2016). O tamanho e a homogeneidade do *spot* é importante para o desempenho da análise (MOAT *et al.*, 2020).

As amostras de plasma são simplesmente deixadas a secar nos cartões de papel filtro, a temperatura ambiente, sem qualquer outro processamento. Os analitos são adsorvidos com os componentes do plasma sobre uma matriz sólida, à base de celulose. A adsorção e a natureza das amostras em papel filtro (DS) tornam os analitos menos reativos do que na matriz fluída, o que geralmente exibe uma excelente estabilidade dos analitos nas condições ambientais, por vários dias e até vários meses, em alguns casos (WAGNER *et al.*, 2016). O pré-tratamento típico de amostras de DS envolve perfuração, extração, centrifugação, transferência de alíquotas, secagem e dissolução em um solvente apropriado (WILHELM; DEN BURGER; SWART, 2014). No entanto, essa metodologia apresenta algumas desvantagens como o risco de contaminação da amostra e o pequeno volume disponível, portanto é necessária uma técnica analítica sensível (WILHELM; DEN BURGER; SWART, 2014). A espectrometria de massas tem sido o método que apresenta a sensibilidade necessária para a medição de concentrações de fármaco em amostras DS com baixa concentração e especificidade necessária para aplicações clínicas (ANTUNES; CHARÃO; LINDEN, 2016).

Independente da metodologia analítica de escolha, os procedimentos desenvolvidos devem atender as mais diversas exigências de aplicações analíticas, assegurando assim a confiabilidade dos resultados obtidos (MORREAU & SIQUIERA, 2016; ANVISA, 2017). Para tanto, todo método desenvolvido deve ser validado de acordo com rígidas especificações técnicas descritas nos guias de validação da área. A validação consiste na realização de vários testes, que incluem a determinação dos seguintes parâmetros: seletividade, limite inferior de detecção e quantificação, linearidade, exatidão e precisão, efeito *carry-over*, efeito matriz e estabilidade da amostra (ANVISA, 2017; EMA, 2011; MOREAU; SIQUEIRA, 2016; PETERS; MAURER, 2002; TSAI *et al.*, 2013).

Diante do exposto, fica evidenciada a importância da monitorização terapêutica de antimicrobianos como a vancomicina na prática clínica o que subsidia o desenvolvimento de técnicas analíticas que sejam mais específicas e capazes de identificar com maior exatidão a concentração destas substâncias no organismo. Sendo assim, a alta sensibilidade e especificidade no desenvolvimento de uma metodologia analítica motiva a busca por técnicas, como LC-MS/MS. Além disso, preconizam-se métodos que utilizem o menor volume de solventes orgânicos, que sejam rápidos, fáceis de serem executados, que exijam um baixo custo e que tenha aplicabilidade na prática clínica de monitoramento terapêutico, podendo contribuir para agilizar o processo de emissão de laudos do Hospital de Pronto Socorro de Porto Alegre (HPS) e em outros laboratórios que prestam este serviço.

3. OBJETIVOS

3.1 OBJETIVO GERAL

Desenvolver e validar uma nova metodologia analítica por LC-MS/MS para a determinação e quantificação de vancomicina em amostras de plasma seco em papel filtro.

3.2 OBJETIVOS ESPECÍFICOS

- Determinar as condições para a detecção, identificação e quantificação plasmática da vancomicina por espectrometria de massas.
- Comparar os resultados da vancomicina obtidos com o método imunoensaios enzimático de multiplicação (EMIT) com os resultados obtidos por DPS-LC-MS/MS.
- Disponibilizar a metodologia proposta para uso da Central Analítica da Universidade Federal de Ciências da Saúde de Porto Alegre e em outros laboratórios que realizem este tipo de análise.

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5. ARTIGO CIENTÍFICO

O manuscrito "*Developmentand validation of a dried plasma spot LC-MS/MS method for the determination of vancomycin and comparison with enzyme-multiplied immunoassay*", que conta com a parte experimental analítica desenvolvida, foi submetido para publicação na revista *Clinica Chimica Acta* (ISSN *0009-8981)* e está apresentado a seguir. As normas de publicação estão disponíveis no Anexo B.

Development and validation of a dried plasma spot LC-MS/MS method for the determination of vancomycin and comparison with enzyme-multiplied immunoassay

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ABSTRACT

Background: Vancomycin is used as antimicrobial agent for the treatment of severe gram-positive infections. The importance of therapeutic monitoring of antimicrobials has led to the development of more specific samples preparations techniques capable of identifying with accuracy the concentration of this substance in the organism.

Methods: An aliquot of 10 µL of plasma was transferred on the paper disk and completely dried under at room temperature. The extraction was performed cutting and transferred the paper to a polypropylene microtube and added sodium phosphate buffer, and internal standard. The mixture was shaken, centrifuge, and a 5 µL aliquot was injected into the UHPLC-MS/MS system.

Results: The optimization was conducted by experimental planning and validated; the intra and inter-day precision values, at the lowest concentration levels, were always less than 20%, considering their relative standard deviation. As for the accuracy values, these were also satisfactory (above 80%). This method was successfully applied to 75 samples of patients undergoing vancomycin therapy.

Conclusion: This work was able to achieve its goals of optimizing, validating the analytical methodology and analyzing plasma samples from patients using vancomycin. The method showed good correlation with the current immunoenzymatic method, and its applicability in the clinical practice of therapeutic monitoring is possible.

*Keywords***:** Dried plasma spots, vancomycin, therapeutic drug monitoring, clinical toxicology, LC-MS/MS.

1. Introduction

Vancomycin is a tricyclic glycopeptide universally considered the drug of choice for initial *empiric* broad-spectrum *antibiotic therapy against* gram-positive bacteria, including *Clostridium difficile*, *Staphylococcus epidermidis*, and methicillin-resistant *Staphylococcus aureus* [1-4]. The antimicrobial effect of vancomycin occurs through the inhibition of the transportation of peptidoglycan precursors to the antimicrobial mechanism for cell-wall biosynthesis upon binding to D-alanyl-D-alanine precursors [5]. Generally, vancomycin monitoring in patients is recommended due to its narrow therapeutic index and toxicity [6]. Underdosing can result in negative prognosis including therapy failure and the occurrence of antibiotic-resistant bacterial strains. Nevertheless, high doses of vancomycin or longer duration of therapy correlates with nephrotoxicity and ototoxicity [6-8]. Thus, maintenance of plasmatic vancomycin concentration in 5–50 mg/L is highly recommended with empiric doses of 15–20 mg/kg (based on actual body weight) administered by intermittent infusion every 8–12 h in patients with normal kidney function [1]. In addition, clinical guidelines recommended maintenance of plasmatic vancomycin concentration in 15–20 mg/L to improve clinical outcomes [9, 10]. However, an increased risk of nephrotoxicity has been reported in these conditions [10-12].

Traditionally, the therapeutic drug monitoring (TDM) was emerged as a tool to evaluate the clinical conditions based in the measuring drug concentration in biological samples [13]. Immunoassays are considered the most popular analytical methodology for the determination of vancomycin due to low cost, simple and straight forward analysis. However, the vancomycin degradation products have been described to interfere with some immunoassays [14]. In addition, it is important to consider the possibility of low specificity promoted by cross-reactivity [15]. In this context, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is proposed as innovative technique to vancomycin TDM. Successful results are described using classical sample preparation methods followed by LC-MS/MS analysis [2,4,14-17].

Dried spot is a biosampling approach widely recommended due to its pre-analytical simplicity once presented several advantages that includes, small volume requirement, increased stability of numerous analytes, easy storage, and transportation [18,19]. Scribel and collaborators development an LC-MS/MS method for quantification of vancomycin in dried spot using whole blood samples, however the correlation with plasma concentration could not be accurately predicted [20]. Probably the estimate of plasma or serum levels from DBS measurements is affected by the distribution of the drug within the blood cells and other parameters [4,20].

Therefore, the aim of this study was the development, optimization and validation of a method for the determination of vancomycin in dried plasma spots (DPS) analyzed by LC-MS/MS. The developed method was successfully applied to 75 samples and its results were compared to the current used immunoassay technique.

2. Material and methods

2.1 Chemicals and Reagents

Vancomycin hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA) and the internal standard (IS) polymyxin B from ICN Biomedicals (IIIkirch-Graffenstaden, France). Both analytical standards were ≥ 98% purity. LC-MS/MS grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Formic acid 98% and sodium phosphate used to prepare the buffer were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water was purified using a Milli-Q system (Millipore, Billerica, MA, USA). Whatman 903® paper was obtained from GE Healthcare (Westborough, USA). Work solutions of both vancomycin and IS were prepared at a concentration of 100 µg/mL in water. When not in use, solutions were stored under appropriate refrigeration (2 - 8 ºC).

2.2 Plasma samples

Blank plasma samples were used for method development and validation and obtained from volunteers who declared not to have used the substance under study. To verify its negativity, an aliquot from each sample was processed and analyzed according to the method proposed.

Plasma samples (n = 75) were obtained from patients under vancomycin treatment in intensive care units of *Pronto Socorro Hospital* (Porto Alegre, Brazil). These samples were previously analyzed by the enzyme-multiplied immunoassay technique (EMIT®). The samples were collected between December 2018 and April 2020. The study protocol was approved by the Research Ethics Committee of the Federal University of Health Sciences of Porto Alegre (Ethics Protocol Approval No. 3.035.913).

2.3 Sample preparation procedure and optimization

An aliquot of 10 µL of plasma was pipetted on the Whatman 903® paper and completely dried under at room temperature. The extraction was performed as follows: the DPS disk (5 mm diameter) was cutted, transferred to a polypropylene microtube and added 250 µL of 200 mM sodium phosphate buffer (pH 5), and 10 µL of IS (20 µg/mL). The mixture was shaken for 35 min at 300 rpm on an orbital shaker. After centrifugation for 5 minutes at 15,000 rpm, a 5 µL aliquot was injected into the LC-MS/MS system.

Moreover, the optimization of the best conditions of extraction solvent was performed through a simplex-centroid design with the solvents water, acetonitrile and methanol. Furthermore, extraction time (10, 20, 30, 40 and 50 minutes) and pH (3, 6 and 9) were investigated using a Doehlert design. All results were evaluated considering the chromatographic peak area of vancomycin. Data obtained in experiments was processed by the software Statistic 8.0 (Statsoft, Tulsa, OK, USA).

2.4 LC-MS/MS analysis and data acquisition

The analyses were performed in a Nexera UFLC system coupled to a LCMS-8040 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The electrospray parameters were set in the positive ion mode as follows: capillary voltage, 4000 V; desolvation line temperature, 250 ºC; heating block temperature, 400 ºC; drying gas, 18 L/min; and nebulizing gas, 2 L/min. Collision-induced dissociation was obtained with 230 kPa argon pressure. Analyses were carried out with multiple reaction monitoring (MRM) by using the *m/z* 725.8 → *m/z* 144.1, *m/z* 725.8 → *m/z* 100.2, and *m/z* 725.8 → *m/z* 83.1 for detection of vancomycin ($[M+2H]^+$); and *m/z* 602.6 → *m/z* 101.1, *m/z* 602.6 → *m/z* 120.1, and *m/z* 602.6 → *m/z* 86.3 for detection of IS ($[M+2H]^+$). The chromatographic separation was achieved with a 75 x 2.0 mm i.d., 2.2 µm, Shim-pack XR-ODS II column (Shimadzu, Kyoto, Japan) eluted with flow rate of 400 µL/min and 50 ºC with a gradient of 0.1% formic acid in water (A) and acetonitrile (B) as follows: 0 – 1.5 min, 5 – 100% of B; 1.5 – 1.6 min, 100 – 5% of B; 1.6 – 5 min, 5% of B. The data were processed using LabSolutions software (Shimadzu, Kyoto, Japan).

2.5 Validation

The method has been validated by establishing parameters, such as the lower limit of quantification (LLOQ), calibration curve, accuracy and precision (within and between-run) [21]. LLOQ was determined by an empirical method, analyzing a series of blank samples and fortified with decreasing amounts of the analyte. The analyses were performed in sextuplicate and the coefficient of variation between replicates should be lower than 20%.

Calibration curve was carried out by the addition of vancomycin in blank samples in seven different concentrations, with six replicates for each concentration. The concentration range was evaluated considering the therapeutic and toxic concentrations of vancomycin (1 to 36 µg/mL).

To perform the accuracy and precision (within and between-run) of the method were evaluated three different quality control (QC) levels: 3 µg/mL for low QC, 15 µg/mL for medium QC and 32 µg/mL for high QC. Experiments were performed in six replicates of each QC, for three consecutive days. Both within and between-run precision values were calculated using one-way analysis of variance (ANOVA).

3. Results and discussion

3.1 Sample preparation optimization

The choice of solvent of extraction in DPS it is a crucial step in the procedure, so in this study the extraction efficiency of water, acetonitrile and methanol was evaluated through a simplex-centroid

design, totalizing 10 experiments, as shown in Table 1. The triangular surface shown in Fig.1 was obtained using the absolute area of the chromatographic response of vancomycin. The highest analytical response was obtained using water as extraction solution (100% of water). To optimize the pH and extraction time parameters, central composite planning was used in two levels of the central point and a sodium phosphate buffer solution range 3,6,9. The results obtained of extraction time and pH was evaluated through a Doehlert design (Table 2). According to the results showed in the Fig. 2 the best results were obtained using pH 5 following by 35 minutes of extraction. These values represent the highest analytical signal intensity for vancomycin and were used in the subsequent validation and analyses. Without statistical analysis, the search for the best extraction parameters could be extended to several empirical experiments, which are time and resource consuming. Also, this statistical approach presents to be more effective, since it makes the evaluation of parameters association possible.

3.2 Validation and proof of applicability

The LLOQ found was considered satisfactory, since it presented an acceptable coefficient of variation and significantly below the therapy concentration, which confirms the applicability of the method. When analyzing the data and the variation coefficient, it was found that the lower limit of quantification of vancomycin is 0.2 µg/mL with the variation coefficient being 17.3%.

After determining the detection limit, the working range was used between 1 µg/mL to 36 µg/mL Then, a linearity was defined in seven different variables in sextuplicate of each concentration. A ratio was calculated between the areas of the vancomycin pattern and IS, obtaining the average for each concentration. The method was considered linear within the stipulated working range. The coefficient of variation for each concentration is among the acceptable variables, smaller 15%. In view of the acceptability of the changes, the linearity graph was constructed. The acceptable correlation coefficient (R^2) must be greater than 0.99, or the value found is $R^2 = 0.994$ for the vancomycin analysis method.

The intra and inter-day precision were evaluated by determining the coefficient of variation of the relative areas displayed among the replicates on three consecutive days. The intra-day variation of the lowest concentration was 12.24%, the average control varied by 12.44%, and the high control by 11.40%. The inter-day variation of the lowest concentration was 7.32%, the average control varied by 7.24%, and the high control by 6.65%. The proposed method reached an accuracy percentage range from 98.9 to 104.8%. Therefore, it is concluded that the method is accurate for determining the concentration of vancomycin.

The developed method was applied to 75 samples of patients treated with vancomycin in the adult, pediatric and burned ICUs, and correlated with the enzyme-multiplied immunoassay technique.

It was possible to conclude that the method proposed in this study has a good correlation with the current method (Fig. 3), Pearson correlation r = 0.9836. Therefore, after a critical analysis of all the

results obtained and the pre-existing information in the literature, it can be concluded that the method of mass spectrometry coupled with liquid chromatography can be applied to clinical practice, with advantages such as: small sample quantity, easy extraction, low solvent cost, high sensitivity.

4. Conclusion

The developed method, the sample preparation is simple, fast, uses small volumes of organic solvents and is a low-cost methodology, as long as there is an initial investment with equipment. The new analytical methodology for the determination and plasma quantification of vancomycin in plasma samples concentrated on filter paper is correlated with the current enzyme-multiplied immunoassay technique, therefore confirming its applicability in clinical practice in the therapeutic monitoring of antimicrobials such as vancomycin.

Acknowledgments

We would like to acknowledge the clinical staff of Hospital Pronto Socorro de Porto Alegre for the sample collection and Shimadzu Brazil for access to the LC-MS/MS system.

Conflict of interests

There are no financial or other relations that could lead to a conflict of interest.

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Tables

Table 1. Triangular surface planning for choosing the solvent (s) or mixture of solvents in the extraction step.

Experiment	Water (%)	Acetonitrile (%)	Methanol (%)	Area
1	100	0	0	16607
2	0	100	0	820
3	0	0	100	11434
4	50	50	0	21453
5	50	0	50	66961
6	0	50	50	5661
7	66,66	16,67	16,67	179928
8	16,67	66,66	16,67	27885
9	16,67	16,67	66,66	43276
10	33,33	33,33	33,33	70249

Table 2. Result of method optimization based on 2 (two) pH variables and extraction time

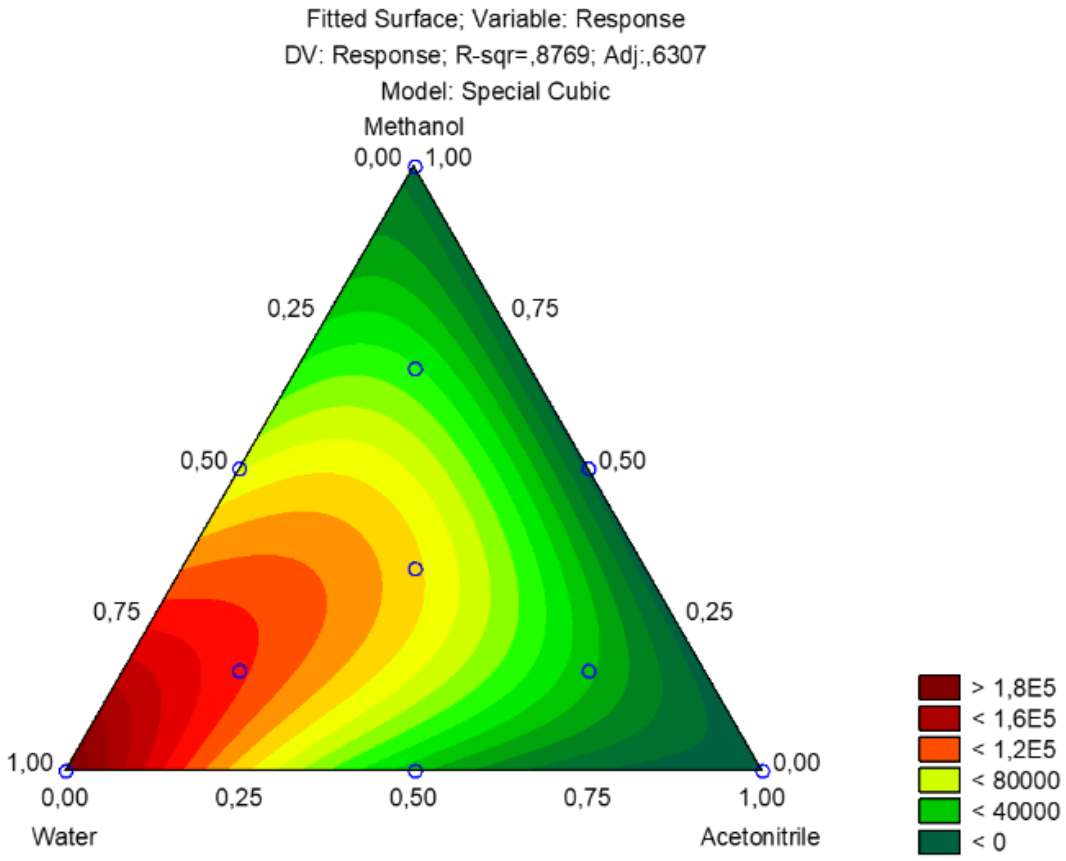
Experiment	pH	Time	Area
1	6	10	133548
2	3	20	180635
3	9	20	114159
4	6	30	237205
5	6	30	253073
6	6	30	244606
7	3	40	238144
8	9	40	131812
9	6	50	222707

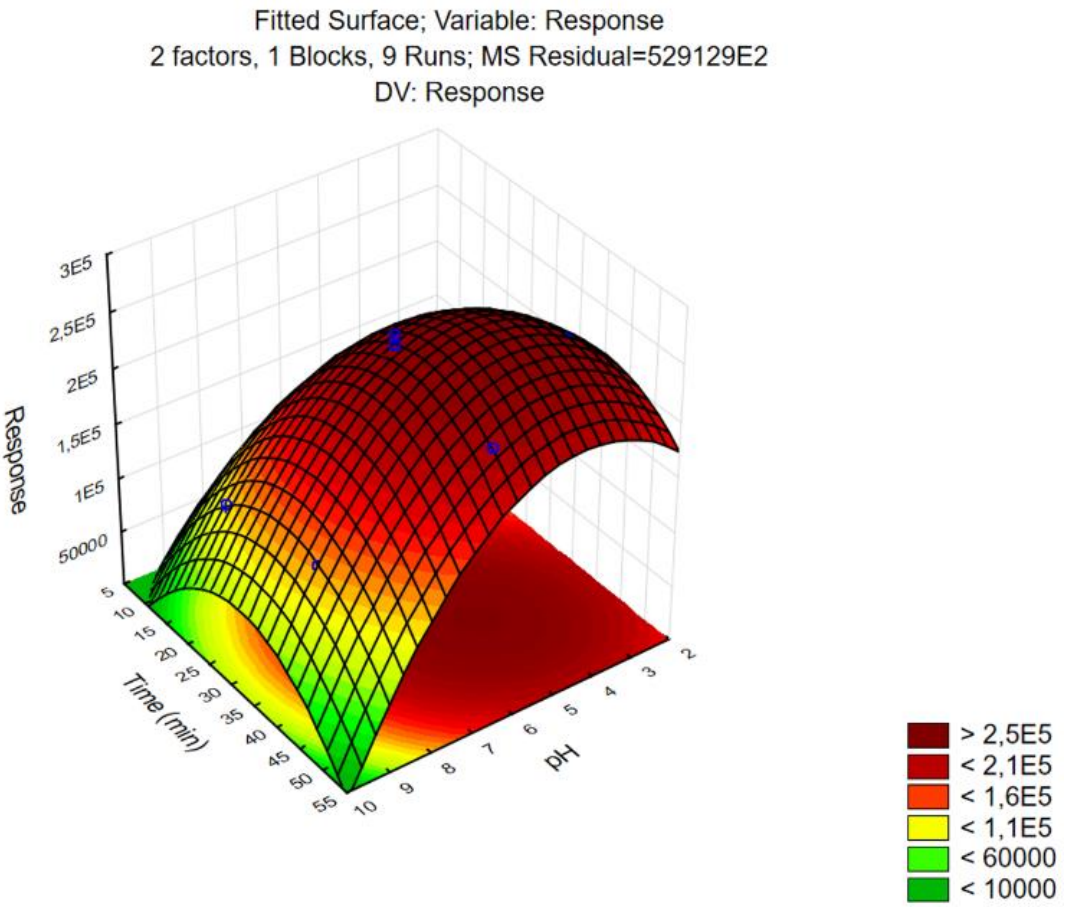
Figures Captions

Fig. 1. Triangular surface obtained from a simplex-centroid design for the optimization of extraction solvent.

Fig. 2. Vancomycin surface response for the evaluation of the extraction time (10, 20, 30, 40 and 50 minutes) and pH (3, 6 and 9).

Fig.3. Pearson correlation of 75 plasma samples analyzed by both DPS-LC-MS/MS and EMIT®.


Fitted Surface; Variable: Response
DV: Response; R-sqr=,8769; Adj:,6307
Model: Special Cubic
Methanol
0,00
1,00
0,25
0,75
0,50
0,50
0,75
0,25
1,00
0,00
0,00
0,25
0,50
0,75
1,00
Water
Acetonitrile
> 1,8E5
< 1,6E5
< 1,2E5
< 80000
< 40000
< 0


Fitted Surface; Variable: Response
2 factors, 1 Blocks, 9 Runs; MS Residual=529129E2
DV: Response
Response
3E5
2,5E5
2E5
1,5E5
1E5
50000
Time (min)
5
10
15
20
25
30
35
40
45
50
55
pH
10
9
8
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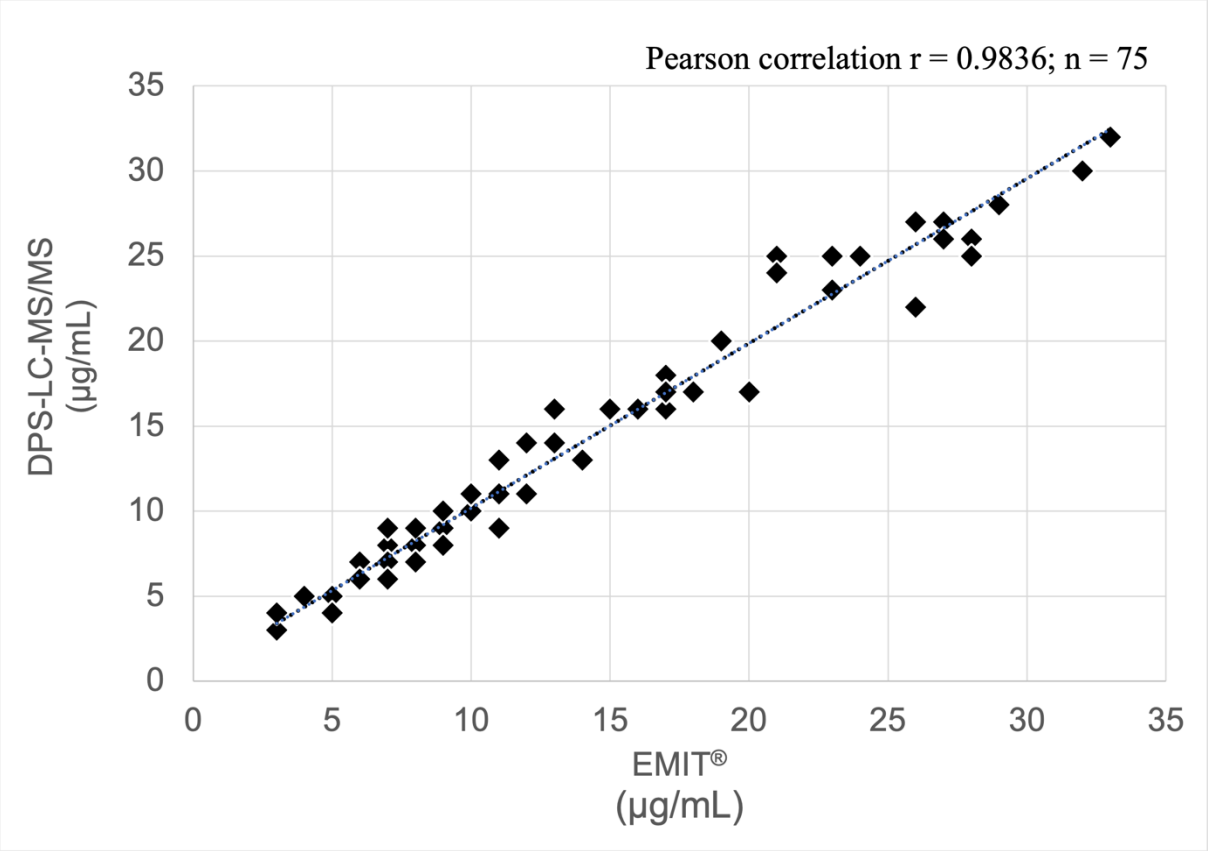
3
2
> 2,5E5
< 2,1E5
< 1,6E5
< 1,1E5
< 60000
< 10000


Pearson correlation r = 0.9836; n = 75
DPS-LC-MS/MS (µg/mL)
EMIT® (µg/mL)
0
5
10
15
20
25
30
35

6. CONCLUSÀO

Neste trabalho foi proposta uma metodologia para determinação de vancomicina em amostras de plasma em papel filtro seco e análise por cromatografia líquida acoplada à espectrometria de massas. O preparo de amostras foi considerado simples, rápido, utilizando pequenos volumes de solventes orgânicos e é uma metodologia de baixo custo, desde que se tenha um investimento inicial com equipamento. A metodologia desenvolvida é correlacionável com a técnica de EMIT, portanto confirma sua aplicabilidade na prática clínica no monitoramento terapêutico de antimicrobianos como a vancomicina.

7. ANEXOS

7.1 ANEXO A. PARECER DA COMISSÃO DE ÉTICA (CEP)

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Dilute-and.-Shoot-HRMS para quantificação de vancomicina em amostras de plasma em papel filtro seco **Pesquisador:** TIAGO FRANCO DE OLIVEIRA **Área Temática:**

Versão: 1

CAAE: 02371218.2.0000.5345

Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.035.913

Apresentação do Projeto:

Trata-se de uma pesquisa realizada em laboratório que pretende desenvolver e validar uma metodologia analítica para determinação e quantificação sérica da vancomicina. A metodologia proposta é a espectrometria de massas de alta resolução (HRMS) através da técnica de concentração de plasma sanguíneo em papel filtro (DPS), extração com solvente adequado e infusão direta em um sistema de ESI-Q -TOF. Pesquisa a ser realizada com resíduos de amostras de plasma (material de descarte) previamente analisadas pelo método imunoenzimático (protocolo vigente) com a finalidade de monitoramento terapêutico de pacientes que recebem vancomicina nas UTIs de adulto, pediátrica e de queimados, do Hospital Pronto Socorro (HPS). Pretendem realizar um estudo piloto para otimizar, validar e aplicar o método. Ao final do estudo, após a conclusão do desenvolvimento experimental da metodologia analítica será realizado a análise e interpretação dos resultados experimentais, para verificar a viabilidade da aplicabilidade do método na prática clínica de monitorização terapêutica, levando-se em conta

características como: simplicidade, rapidez, custos e a utilização de um menor volume de solventes orgânicos, para que assim possa ser implantado na rotina de laboratórios.

Objetivo da Pesquisa:

Desenvolver e validar uma metodologia analítica para a determinação e quantificação sérica da vancomicina, que tenha aplicabilidade na prática clínica de monitorização terapêutica.

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:**90.050-170
UF: RS **Município:**PORTO ALEGRE
Telefone:(51)3303-8804 **E-mail:**cep@ufcspa.edu.br

Continuação do Parecer: 3.035.913

Avaliação dos Riscos e Benefícios:

Riscos: o risco é que com o método proposto (HRMS) não se obtenha resultados quanto a sensibilidade, especificidade, rapidez, custo baixo que justifique sua implantação em uma rotina laboratorial.
Benefícios: desenvolver uma metodologia que utilize um menor volume de solventes orgânicos, que seja rápido, fácil de ser executado, que exija um baixo custo e que tenha aplicabilidade na prática clínica de monitoramento terapêutico, contribuindo para agilizar o processo de emissão de laudos.

Comentários e Considerações sobre a Pesquisa:

Solicita dispensa do uso de TCLE visto que serão utilizados resíduos de amostras de plasma previamente analisadas pelo laboratório do HPS, que seriam descartadas.

Considerações sobre os Termos de apresentação obrigatória:

Apresentam todos o Termos obrigatórios: 1.Termo Autorização Liberação Amostras; 2.Termo de Anuência da UFCSPA; 3.Termo de Anuência do Responsável pelo Setor HPS; 4.Termo de Ciência e Autorização do HPS; 5.Termo de Compromisso de Utilização e Divulgação dos Dados; 6.Termo de Compromisso Entrega de Relatórios.

Recomendações:

Sugere-se acrescentar o Projeto em formato word na PB.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1234968.pdf	30/10/2018 22:49:57		Aceito
Orçamento	Orcamento_do_projeto_de_pesquisa.pdf	30/10/2018 22:49:13	FERNANDA RIBEIRO VIDAL	Aceito
Outros	Autorizacao_de_liberacao_de_amostras_de_descarte_do_HPS.jpg	30/10/2018 22:36:52	FERNANDA RIBEIRO VIDAL	Aceito
Outros	Termo_de_compromisso_da_utilizaca	30/10/2018	FERNANDA	Aceito

Outros	o_dos_dados.jpg	22:28:44	VIDAL	Aceito
Cronograma	Cronograma_do_projeto_de_pesquisa.pdf	30/10/2018 22:26:29	FERNANDA RIBEIRO VIDAL	Aceito
Declaração de Pesquisadores	Termo_de_compromisso_da_entrega_de_relatorios.jpg	30/10/2018 22:17:47	FERNANDA RIBEIRO VIDAL	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Justificativa_de_ausencia.pdf	30/10/2018 22:13:46	FERNANDA RIBEIRO VIDAL	Aceito
Declaração de Instituição e	Termo_de_anuencia_responsavel_UFCSPA.jpg	30/10/2018 22:13:13	FERNANDA	Aceito

Infraestrutura			RIBEIRO VIDAL	
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Outros	Termo_de_anuencia_pelo_responsavel_ HPS.jpg	30/10/2018 22:02:00	FERNANDA RIBEIRO VIDAL	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_pesquisa.pdf	30/10/2018 21:17:19	FERNANDA RIBEIRO VIDAL	Aceito
Folha de Rosto	Folha_de_Rosto.pdf	30/10/2018 10:14:19	FERNANDA RIBEIRO VIDAL	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

PORTO ALEGRE, 23 de Novembro de 2018

Assinado por:

Fernanda Bordignon Nunes

(Coordenador(a))

7.2 ANEXO B. NORMAS DE PUBLICAÇÃO DA REVISTA *CLINICA CHIMICA ACTA*

CLINICA CHIMICA ACTA

International Journal of Clinical Chemistry and. Diagnostic Laboratory Medicine

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0009-8981

DESCRIPTION

*Clínica Chimica Act a*si a high si jornal chicha Publisher original Ressarce Communications in lhe Field off **clínica clínica** and.**laboratory medicine**, defined as the **diagnostic** application of chemistry, biochemistry, immunochemistry, biochemical aspects of hematology, toxicology, and. Molecular biology to the study of human disease in body fluids and. cells.

The objective of the journal is to publish novel information leading to a better understanding of biological mechanisms of human diseases, their prevention, diagnosis, and. patient management. Reports of an applied clinical character are also welcome. Papers concerned with normal metabolic processes or with constituents of normal cells or body fluids, such as reports of experimental or clinical studies in animals, are only considered when they are clearly and directly relevant to human disease. Evaluation of commercial products have a low priority for publication, unless they are novel or represent a technological breakthrough. Studies dealing with effects of drugs and natural products and studies dealing with the redox status in various diseases are not within the journal's scope. Development and evaluation of novel analytical methodologies where applicable to diagnostic clinical chemistry and laboratory medicine,

including point-of-care testing, and topics on laboratory management and informatics will also be considered. Studies focused on emerging diagnostic technologies and (big) data analysis procedures including digitalization, mobile Health, and artificial Intelligence applied to Laboratory Medicine are also of interest.

AUDIENCE

Medical biochemists, clinical chemists, analytical chemists.

IMPACT FACTOR

2018: 2.735 © Clarivate Analytics Journal Citation Reports 2019

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- E-mail address
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All necessary files have been uploaded:

Manuscript:

- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)

Supplemental files (where applicable)

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Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example

Authorship

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