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ALBERTO GOMES TAVARES JUNIOR

Desenvolvimento e validação de método por CLUE-EM/EM para monitoramento plasmático de Losartana e seu metabólito ativo em pacientes com doença renal crônica

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal do Amapá para obtenção do Título de Mestre em Ciências Farmacêuticas.

Orientador: Francisco Fábio Oliveira de Sousa
Co-orientador: Lílian Grace da Silva Solon

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Programa de Pós-Graduação em Ciências Farmacêuticas

da Universidade Federal do Amapá

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SUMÁRIO

DESENVOLVIMENTO E VALIDAÇÃO DE MÉTODO POR CLUE-EM/EM PARA MONITORAMENTO PLASMÁTICO DE LOSARTANA E SEU METABÓLITO ATIVO EM PACIENTES COM DOENÇA RENAL CRÔNICA

RESUMO

Introdução: O monitoramento terapêutico de Losartana e seu metabólito por métodos bioanalíticos em pacientes renais crônicos visa promover a individualização posológica e a otimização dos tratamentos farmacológicos, além de prevenir terapias invasivas e garantir a segurança do paciente. **Objetivo:** Assim, o objetivo foi avaliar o perfil farmacoterapeutico dos pacientes renais amblatorias de um hospital público no amapá, além de desenvolver e validar um método por Cromatografia Líquida de Ultra Eficiência acoplada a Espectroscopia de Massas sequencial (CLUE-EM/EM) para quantificação simultânea de Losartana e seu metabólito ativo para monitoramento plasmático em pacientes renais crônicos. **Metodologia:** No primeiro momento, levantou-se dados de comorbidades e medicamentos utilizados pelos pacientes, resultando como Hipertensão a comorbidade principal e Losartana como medicamento de maior frequência de uso, sendo o mesmo e seu metabólito ativo (Ácido Losartana) selecionados para o desenvolvimento de método capaz de monitorar suas concentrações em plasma humano. Realizou-se a coleta de sangue de cinco paciente renais crônicos que faziam uso de Losartana. O método desenvolvido foi validado analiticamente e bioanaliticamente para a matriz plasma. **Resultados e discussões:** O método apresentou linearidade, precisão, exatidão, sensibilidade, robustez, eficiência de extração e seletividade para os intervalos de concentração de 0,005 – 1 µg/mL para Losartana e de 0,001 – 6 µg/mL para o Ácido Losartana. Na aplicação do método nas amostras dos pacientes, observou-se que dois pacientes apresentaram níveis subterapêuticos de Losartana e do metabólito após a administração da dose de manutenção, demonstrando a necessidade de monitorar este anti-hipertensivo administrado aos pacientes renais, para o controle da pressão arterial, e garantir eficácia e segurança no tratamento farmacológico. **Conclusão**: Portanto, se apresentou um método eficaz e sensível, possibilitando a implementação de protocolos clínicos para o monitoramento da Losartana

Palavras-Chave: Doença Renal Crônica; Monitoramento terapêutico de farmácos; CLUE-EM/EM; Losartana; Ácido Losartana.

Agradecimentos: CAPES, FAPEAP (PPSUS), USC-Spain, PPGCF-UNIFAP

ABSTRACT

DEVELOPMENT AND VALIDATION OF AN UHPLC-MS/MS METHOD FOR THE PLASMATIC MONITORING OF LOSARTAN AND ITS ACTIVE METABOLITE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

Introduction: The therapeutic monitoring of Losartan and its metabolite by bioanalytical methods in chronic renal patients aims to promote the posologic individualization and optimization of pharmacological treatments, as well as to prevent invasive therapies and to guarantee patient safety. **Objective**: The aim of this study was to evaluate the pharmacotherapeutic profile of ambulatory kidney patients at a public hospital in amapá, in addition to developing and validating a method by Liquid Chromatography of Ultra Efficiency coupled to sequential Mass Spectroscopy (UHPLC-MS/MS) for quantification of Losartan and its active metabolite for plasmatic monitoring in kidney chronic patients. **Methodology**: At the first moment, we obtained data on comorbidities and medications used by the patients, resulting in hypertension as main comorbidity and Losartan as the most frequently used medication, being the same and its active metabolite (Losartan Acid) selected for the development of method able to monitor their concentrations in human plasma. Blood was collected from five chronic renal patients who were taking Losartan. The developed method was validated analytically and bioanalytically for the plasma matrix. **Results and discussion**: The method presented linearity, precision, accuracy, sensitivity, robustness, extraction efficiency and selectivity for the concentration ranges from 0.005 - 1 µg/mL for Losartan and 0.001 - 6 µg/mL for Losartan. In the application of the method in the patient samples, it was observed that two patients presented subtherapeutic levels of Losartan and the metabolite after administration of maintenance dose, demonstrating the need to monitor this antihypertensive administered to renal patients for control of blood pressure, and ensure efficacy and safety in pharmacological treatment. Conclusion: Therefore, efficient and sensitive method was presented, enabling the implementation of clinical protocols for the monitoring of Losartan

Keywords: Chronic Kidney Disease; Therapeutic drug monitoring; UHPLC-MS/MS; Losartan; Losartan acid.

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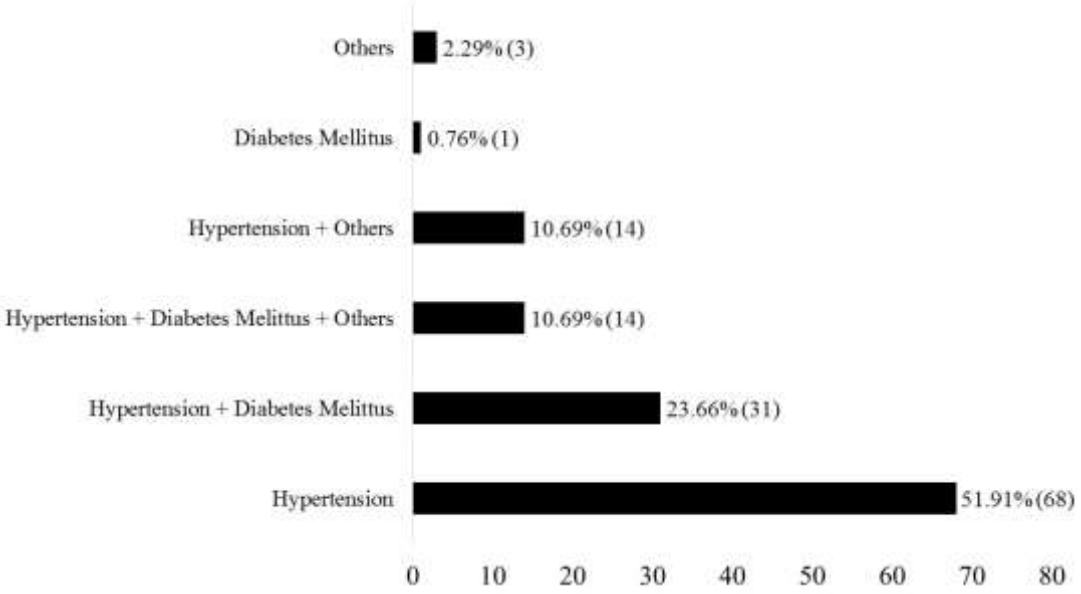
1 INTRODUÇÃO

1.1. DOENÇAS RENAIS

Os rins são órgãos responsáveis pela regulação fisiológica do corpo humano, tais como pressão sanguínea, osmolaridade, pH, balanço iônico, excretar resíduos e substâncias estranhas, dentre outros (SILVERTHORN, 2010). Quando o rim apresenta danos severos, comprometendo suas funções fisiológicas, o paciente pode apresentar o quadro clínico de insuficiência renal, sendo classificada como aguda ou crônica (GAGNEBIN et al., 2018; CAÑADAS-GARRE et al., 2018).

A insuficiência renal aguda apresenta uma redução, na função dos rins, acelerada, mantendo-se por períodos variáveis. Dependendo do acompanhamento e terapia empregada, este quadro clínico pode ser reversível. Já insuficiência renal crônica é caracterizada pela perda lenta, progressiva e irreversível das funções renais, podendo ser até certo ponto assintomáticas ou não (SILVERTHORN, 2010; GAGNEBIN et al., 2018; CAÑADAS-GARRE et al., 2018).

A doença renal crônica (DRC) se tornou um grave problema de saúde pública devido à sua comorbidade com doenças crônicas como hipertensão arterial sistêmica e diabetes mellitus. Silva (2017), realizou um estudo com 156 pacientes renais crônicos na Unidade de Nefrologia do HCAL, onde observou-se que 83,79 % apresente pelo menos uma comorbidade conforme a **Figura 1**.



Reproduzido com a permissão de SILVA, 2017. Legenda: *Hypertension* (Hipertensão), *Others* (Outros).

1.2. LOSARTANA POTÁSSICA

A Losartana é um composto sintético potente, anti-hipertensivo, ativo por via oral, considerado o primeiro antagonista do receptor da angiotensina II (ARA II), não peptídico. Mais especificamente, trata-se de um antagonista total, competitivo e específico dos receptores da angiotensina II, metabolizado em ácido Losartana (EXP3174), que é uma molécula química similar e com maior potencial terapêutico, sedo 10 a 40 vezes mais potente do que a Losartana (LO., 1995; AL-MAJED., 2015)

1.2.1. Caracteristicas Farmacocinética

A losartana é um fármaco de rapida absorção por via oral, atingindo pico de concentração plasmática máximo (C_{max} = 252,6 ± 102,6 ng/ml) de 1 a 2 horas após a administração (T_{max} = 1,36 ± 0,71), com tempo de meia-vida de 2 horas ($T_{1/2}$ = 2,84 ± 1,14).

O volume de distribuição da losartana é de 34 litros, ambas as moléculas têm ligação às proteínas plasmáticas maior que 99%, com concentrações livres para os órgãos-alvo nos locais de seus receptores (LO., 1995; AL-MAJED., 2015).

Aproximadamente 14% de uma dose de losartana é convertida no metabótito EXP3174, este atingi pico de concentração plasmática (C_{max} = 283,2 ± 127,1 ng/ml) de 3 a 4 horas (T_{max} = 3,80 ± 1,20) e sua meia-vida de 6 a 9 horas ($T_{1/2}$ = 6,01 ± 0,94). A principal via metabólica para a losartana é através do citocromo P450 (LO., 1995; AL-MAJED., 2015).

Em relação a área abaixo da curva ($AUC_{0\ t}$), a losartana apresenta 480,8 ± 162,6 ng/ml.h e o metabólito 2148,2 ± 853,5 ng/ml.h. A depuração plasmática da losartana e de seu metabólito, aproxima-se de 600mL/min e 50mL/min, respectivamente. A depuração renal é de 74mL/min para losartana e de 26mL/min para EXP3174. Quando o fármaco é administrado por via oral, 4% da dose é excretada na urina e 6% na forma metabolizada (LO., 1995; AL-MAJED., 2015).

1.3. MONITORAMENTO TERAPÊUTICO DE FÁRMACOS

As técnicas de monitoramento terapêutico de fármacos (MTF) são empregadas para garantir a segurança e eficácia de um tratamento farmacológico e nos estudos de farmacocinética. A sua implementação possibilita a otimização do custo-benefício associado à intervenção medicamentosa (TOUW et al., 2005).

Justifica-se a sua aplicação a fármacos com margem terapêutica estreita, fármacos com elevada variabilidade farmacocinética e em grupos de pacientes com características farmacocinéticas particulares, como neonatos ou renais. É também relevante o MTF de fármacos sem especificidade clínica de eficácia ou toxicidade. Isto é, quando o objetivo clínico é a prevenção ou a ausência de sintomas, em curto prazo só é possível garantir que

esse efeito advém da terapêutica através da certificação por MTF da existência e manutenção de concentrações plasmáticas eficazes. Quando o paciente apresenta sinais e sintomas de toxicidade inespecíficos, por exemplo, distúrbios gastrointestinais numa intoxicação por digitálicos, o estabelecimento de um nexo de causalidade com a terapêutica pode ser estabelecido pela verificação da existência de concentrações plasmáticas excessivas (STOTT; HOPE, 2017; RICHARDS et al., 2017).

Será candidato a monitoramento terapêutica, o fármaco em que não é seguro estabelecer uma relação dose-resposta, mas em que exista uma relação concentração plasmática-resposta farmacológica que permita o ajuste posológico (COUCHMAN et al., 2017; GILS, 2017).

A qualidade dos resultados obtidos pelo monitoramento terapêutico depende da técnica de amostragem, da validação das metodologias analíticas e bioanaliticas, do conhecimento prévio das características farmacocinéticas dos fármacos e da definição de janelas terapêuticas, idealmente através de ensaios clínicos controlados e randomizados, que permitam conhecer as condições ideais de amostragem (LESOSKY; JOSKA; DECLOEDT, 2017; SCHMITZ et al., 2017).

O desenvolvimento e validação de métodos bioanalíticos empregados na determinação quantitativa de fármacos e seus metabólitos em fluidos biológicos desempenha um papel significativo da avaliação e interpretação da biodisponibilidade, bioequivalência, farmacocinética e na obtenção de dados toxicocinéticos do fármaco e seus metabólitos. A qualidade destes estudos está diretamente relacionada à qualidade do método bioanalítico (DA SILVA, 2014).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Desenvolver e validar um método por Cromatografia Líquida de Ultra Eficiência acoplada a Espectroscopia de Massas sequencial (CLUE-EM/EM) para quantificação simultânea de Losartana e seu metabólito ativo para monitoramento plasmático de pacientes renais crônicos.

2.2 OBJETIVOS ESPECÍFICOS

- Determinar e avaliar o perfil farmacoterapêutico e clínico dos pacientes renais entrevistados;
- Validar analiticamente e bioanaliticamente um método por Cromatografia Líquida de Ultra Eficiência acoplada a Espectroscopia de Massas sequencial (CLUE-EM/EM) para quantificação simultânea de Losartana e seu metabólito ativo;
- Aplicar o método desenvolvido em amostras reais de pacientes renais crônicos;

3 ARTIGO 1

Pretensão de submissão no Journal of Brazilian Journal of Nephrology

Brief communication

Perfil farmacoterapêutico de pacientes renais crônicos ambulatoriais de um hospital público do Estado do Amapá

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Resumo

Introdução: Estima-se que o número de doentes renais crônicos adultos no Brasil esteja entre 11 e 22 milhões. A terapia farmacológica para a DRC envolve o uso de diferentes medicamentos, tornando os pacientes passíveis de experimentar eventos adversos. Determinar o perfil medicamentoso desses pacientes é fundamental para desenvolver medidas de prevenção de reações adversas, reduzir as interações medicamentosas e promover o seu uso racional. **Objetivo:** Avaliar o perfil sociodemográfico e medicamentoso de pacientes renais crônicos no ambulatório em um hospital público do Estado do Amapá. **Métodos**: Neste estudo observacional, descritivo e transversal foram incluídos 43 pacientes diagnosticados com doença renal crônica, sem indicação de hemodiálise. Foram realizadas entrevistas individuais com aplicação de questionário para levantar dados socioeconômicos, comorbidades e perfil de utilização de medicamentos. **Resultados**: O sexo feminino foi predominante (69,8%). Os pacientes apresentaram idade média de 53,4 anos. As comorbidades mais prevalentes foram à hipertensão arterial (79,06%) e o diabetes mellitus (41,86%). Uma média de uso de 2,67 fármacos/paciente foi observada. Cerca de 82,15% dos fármacos utilizados apresentavam riscos para a progressão da doença renal, sendo os anti-hipertensivos os que apresentaram maior frequência de uso. **Conclusão**: O uso de fármacos com potencial nefrotóxico foi predominante, demonstrando a importância do monitoramento farmacoterapêutico personalizado para estes pacientes, como forma de reduzir os riscos e a progressão da doença renal.

Palavras-chave: medicamentos; doença renal crônica; comorbidades; diabetes mellitus; hipertensão arterial

Introdução

A doença renal crônica (DRC) se tornou um problema de saúde pública preocupante, uma vez que apresenta uma prevalência crescente e está diretamente correlacionada com a Diabetes Mellitus e a Hipertensão Arterial.[1]

No Brasil, estima-se que entre 11 e 22 milhões de indivíduos adultos apresentem disfunção renal em diferentes graus, considerando uma população com cerca de 200 milhões de habitantes.[2]

A disfunção dos rins pode gerar danos sistêmicos à saúde do paciente, o que justifica a necessidade de seguimento clínico personalizado, associado às precauções cotidianas que evitem agravos aos quadros clínicos. Quando a função renal do paciente se encontra extremamente comprometida, é necessário que o mesmo se submeta a tratamentos como a hemodiálise, diálise peritoneal ou transplante renal.[3]

A terapia renal substitutiva não é a única forma de tratamento para os casos de doença renal crônica, conforme a fase da doença o paciente é submetido a terapias farmacológicas. A terapia farmacológica para a DRC contempla diversos tratamentos, tais como o hormonal e o uso de fármacos para o tratamento das comorbidades associadas, o que quase sempre leva os pacientes a fazerem uso de diferentes medicamentos, sendo bastante passíveis de experimentar interações medicamentosas e eventos adversos.[4]

O conhecimento do perfil medicamentoso é fundamental para desenvolver medidas de prevenção de reações adversas e promover o uso racional de medicamentos entre os pacientes renais, reduzindo assim o risco de progressão da lesão renal. Neste sentido, o presente estudo objetivou determinar o perfil farmacoterapêutico de pacientes renais crônicos ambulatoriais atendidos em um hospital público do Estado do Amapá.

Métodos

Delimitações do estudo

Tratou-se de um estudo observacional, descritivo e transversal. Foram incluídos todos os pacientes diagnosticados com doença renal crônica (DRC), maiores de 18 anos, sem indicação de hemodiálise, atendidos no ambulatório do Hospital de Clínicas Dr. Alberto Lima (HCAL), que aceitaram participar do estudo por meio da assinatura do Termo de Consentimento Livre e Esclarecido (TCLE).

Coleta de Dados

Para coleta de dados, foram realizadas entrevistas individuais mediante aplicação de questionário adaptado a partir do instrumento *Brief Medication Questionnaire*[5], com o intuito de levantar dados socioeconômicos (sexo, idade, escolaridade, renda, número de moradores por domicílio), dados relacionados à DRC e comorbidades associadas, assim como o perfil de utilização de medicamentos. As entrevistas foram realizadas no período de Fevereiro a Maio de 2018.

Todos os dados foram registrados e analisados por meio de estatística descritiva univariada no software Microsoft Excel *for Windows* 2013. Os medicamentos mencionados nas entrevistas foram agrupados de acordo com a classificação da *Anatomical Therapeutic Chemical* (ATC) do WHO *Collaborating Center for Drug Statistics Methodology*.[6]

Aspectos éticos

Este estudo foi submetido ao Comitê de Ética em Pesquisa da Universidade Federal do Amapá (UNIFAP), sendo registrado com o Certificado de Apresentação de Apreciação Ética (CAAE) nº 76695717.0.0000.0003.

Resultados

No total, 43 pacientes foram incluídos no estudo, dos quais 30 foram mulheres e 13 homens, com idade média de 53,4 anos (mínimo de 18 e máximo de 85 anos). A maior parte dos pacientes apresentava ensino médio completo 30, 2%e renda mensal entre 1 e 3 salários mínimos. As características socioeconômicas dos pacientes estão listadas na **Tabela 1**.

Tabela 1 Características socioeconômicas dos pacientes renais crônicos no ambulatório em um hospital público no Amapá (n=43), 2018

Características	**Número de Pacientes**	**%**
Sexo		
Feminino	30	69,8
Masculino	13	30,2
Faixa etária		
< ou igual a 39	7	16,3
40 a 59	22	51,2
> ou igual a 60	14	32,6
Escolaridade		
Analfabeto	6	14,0
Fundamental completo	4	9,3
Fundamental incompleto	11	25,6
Médio completo	13	30,2
Médio incompleto	4	9,3
Superior completo	3	7,0
Superior incompleto	2	4,7
Renda		

< 1	13	30,2
1 - 3	20	46,5
3 - 5	7	16,3
> 5	0	0,0
Não soube informar	3	7,0

Durante a aplicação do questionário, foi perguntado aos pacientes sobre o seu nível de conhecimento acerca da DRC e 44% respondeu saber pouco sobre a sua condição de saúde.

Todos os pacientes entrevistados afirmaram ter outro diagnóstico, além da DRC. Dentre as doenças relatadas, destacou-se a Hipertensão arterial e a Diabetes Mellitus, principais comorbidades associadas à DRC.[1,7,8] A distribuição das comorbidades associadas à DRC dos pacientes entrevistados encontra-se na **Figura 1**.

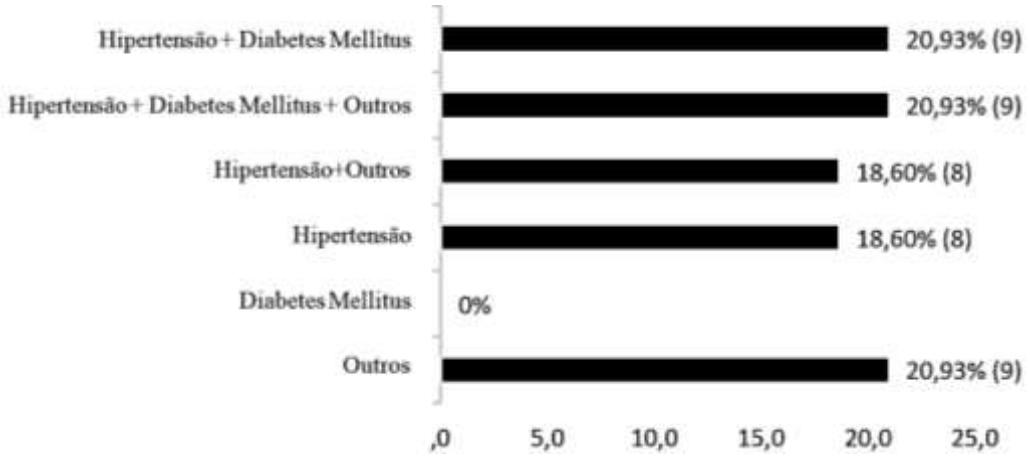

Figura 1 Distribuição das comorbidades associadas à doença renal nos pacientes renais crônicos ambulatoriais (n=43).

Uma média de 2,67 fármacos eram utilizados por paciente. Do total, 28 (65,1%) indivíduos estavam utilizando 2 ou mais medicamentos, dos quais 5 (11,6%) faziam uso de cinco ou mais medicamentos. A distribuição dos medicamentos utilizados pelos pacientes entrevistados encontra-se na **Tabela 2**, apresentando anti-hipertensivos em sua maioria, seguido pelos antidiabéticos.

Tabela 2 Medicamentos utilizados pelos pacientes renais crônicos ambulatoriais entrevistados.

Fármaco	**ATC**	**Frequência (%)**	**n**
Losartana*	C09CA01	15,79	18
Prednisona*	A07EA03	5,26	6
Furosemida*	C03CA01	4,39	5
Hidroclorotiazida*	C03AA03	4,39	5
Ramipril*	C09AA05	4,39	5
Valsartana*	C09CA03	4,39	5
Ácido Acetilsalicílico*	B01AC06	3,51	4
Anlodipina	C09AA05	3,51	4
Atenolol*	C07AB03	3,51	4
Insulina	A10A	3,51	4
Metformina*	A10BA02	3,51	4
Nifedipino*	C08CA05	3,51	4
Propatilnitrato	C01DA07	2,63	3
Sinvastatina*	C10AA01	2,63	3
Citrato de Potássio	A12BA02	1,75	2
Enalapril*	C09AA02	1,75	2
Glibenclamida*	C09AA02	1,75	2
Omeprazol*	A02BC01	1,75	2
Sitagliptina	A10BH01	1,75	2
Outros		26,32	30
Total		**100**	**84**

Legenda: (*) potencial elevado de reações adversas e/ou controle mais rigoroso na dose prescrita para pacientes diagnosticados com doença renal crônica.

Discussão

No que se refere ao gênero, observou-se que a maioria dos pacientes deste grupo foi do sexo feminino, ao contrário do estudo de Silva (2017) realizado com pacientes renais

crônicos dialíticos no Serviço de Nefrologia do mesmo hospital, no qual o predomínio foi do sexo masculino, podendo indicar um maior autocuidado ou ainda a melhor aderência terapêutica por parte do público feminino, evitando assim a progressão da lesão renal a ponto de chegar à terapia renal substitutiva.[1,4]

Em relação às comorbidades associadas à DRC, a hipertensão arterial e a diabetes mellitus foram as mais prevalentes, consideradas de risco elevado para progressão da DRC se não tratadas adequadamente. O estudo de Marquito *et al* (2014), constatou que dentre os 558 pacientes entrevistados, 178 eram diabéticos e 382 hipertensos, confirmando que estas doenças são fatores de risco fortemente associados a DRC.[8]

Dentre os 19 fármacos mais utilizados (**Tabela 2**), 14 deles requeriam monitoramento clínico devido ao potencial elevado de reações adversas e/ou controle mais rigoroso na dose prescrita para pacientes diagnosticados com doença renal crônica em função do risco de nefrotoxicidade, o que representa 82.15% (69) dos fármacos amostrados. Neste grupo estão presentes medicamentos como a losartana (18), furosemida (5), hidroclorotiazida (5), ramipril (5), valsartana (5) e ácido acetilsalicílico (4), notoriamente fármacos utilizados no tratamento anti-hipertensivo, uma das principais doenças de base e também consequência da DRC. Podemos comparar este perfil farmacoterapêutico com os achados de Silva (2017), que dentre os 61 diferentes fármacos utilizados pelos pacientes renais dialíticos, 29 deles apresentavam potencial elevado de reações adversas e nefrotoxicidade, o que representa 27.79% (132) dos fármacos amostrados. Fármacos como losartana (61), valsartana (20), ácido acetilsalicílico (12), propranolol (6) e captopril (3) foram os mais utilizados, demonstrando mais uma vez o predomínio no tratamento com anti-hipertensivos.[1]

Locatelli *et al* (2015) apresentou dados semelhantes ao do presente estudo, sendo que do total de 663 pacientes renais crônicos, 36,5% utilizavam fármacos voltados ao aparelho cardiovascular (incluindo os anti-hipertensivos), corroborando assim com os

achados apresentados na **Figura 1**, no que se observa que 79,08% dos pacientes foram diagnosticados com hipertensão arterial.[4]

Conclusão

Os resultados obtidos revelaram o perfil sociodemográfico e farmacoterapêutico dos pacientes renais crônicos ambulatoriais atendidos pelo sistema público de saúde do Estado do Amapá, observando que as maiores quantidades dos medicamentos utilizados eram decorrentes das comorbidades associadas. Os pacientes entrevistados necessitam de um monitoramento farmacológico diferenciado, visto que o uso de fármacos com potencial nefrotóxico é predominante e que se for conduzido de forma irracional pode ocasionar a progressão da doença, necessitando inevitavelmente submeter o paciente à terapia renal substitutiva. A partir dos achados, destaca-se a necessidade de aprofundar os estudos visando identificar e sugerir alternativas farmacológicas e contornar as eventuais interações medicamentosas existentes a partir do seguimento farmacoterapêutico, reduzindo assim os riscos relacionados aos medicamentos utilizados pelos pacientes com doença renal crônica.

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Referências

1. Silva UD de A. Avaliação dos riscos associados à farmacoterapia e impacto do seguimento farmacoterapêutico de pacientes renais crônicos do Estado do Amapá. Universidade Federal do Amapá; 2017.

2. Sarmento LR, Fernandes PFCBC, Pontes MX, Correia DBS, Chaves VCB, Arnaud TL, et al. Prevalência das causas primárias de doença renal crônica terminal (DRCT) validadas clinicamente em uma capital do Nordeste brasileiro. Braz J Nephrol. 2018;40(2):130–5.

3. Silverthorn DU. Fisiologia Humana: uma abordagem integrada. fifth ed. Artmed, editor. Artmed. Porto Alegre: Artmed; 2010. 1-867 p.

4. Locatelli C, Spanevello S. Perfil medicamentoso de pacientes sob tratamento de terapia renal substitutiva em um Hospital do Rio Grande do Sul. Rev Soc Bras Clin Med. 2015;13(3):240–5.

5. Ben AJ, Neumann CR, Mengue SS. Questionnaire and Morisky- Green Test to evaluate. Rev Saúde Pública. 2012;46(2):1–10.

6. WHO. ATC - Structure and principles [Internet]. WHO Collaborating Centre for Drug Statistics Methodology. 2012 [cited 2019 Feb 25]. p. 9–12. Available from: http://www.whocc.no/atc/structure_and_principles/

7. van der Nagel BCH, Versmissen J, Bahmany S, van Gelder T, Koch BCP. High-throughput quantification of 8 antihypertensive drugs and active metabolites in human plasma using UPLC–MS/MS. J Chromatogr B [Internet]. 1st ed. Elsevier B.V.; 2017 Aug;1060(1):367–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1570023217304981

8. Marquito AB, Fernandes NMS, Colugnati FAB, Paula RB. Interações medicamentosas potenciais em pacientes com doença renal crônica. J Bras Nefrol. 2014;36(1):26–34.

4 ARTIGO 2

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Full length article

Development and validation of an UHPLC-MS/MS method for simultaneous plasmatic quantification of Losartan and its active metabolite (EXP3174) in chronic kidney disease patients

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Abstract

This study aimed to validate a method for monitoring Losartan and its active metabolite by ultra-high performance liquid chromatography-tandem mass spectrometry in patients with chronic kidney disease (CKD). Five patients diagnosed with CKD under losartan antihypertensive treatment attending consultations were consensually selected and invited to take part on the study. The spectroscopic method was analytic and bioanalytically validated for plasma matrix, presenting linearity, precision, accuracy, sensitivity, robustness, extraction efficiency and selectivity for concentrations ranging from 0.005 – 1.000 and 0.001 – 6.000 µg / ml for Losartan and Losartan Acid (EXP3174), respectively. The plasma quantification evidenced that 40% of the patients presented subtherapeutic levels of Losartan and its metabolite before and/or after their usual intake, reinforcing the need of monitoring in order to ensure efficacy and safety in their treatments. Thus, the method was validated and could be used to implement safer clinical protocols for kidney disease patients under losartan treatment.

Keywords: chronic kidney disease; therapeutic drug monitoring; UHPLC-MS/MS, losartan, exp3174

1. Introduction

Chronic kidney disease (CKD) has become a major public health concern due to the increasing prevalence and their correlation with Diabetes Mellitus and Hypertension [1]. CKD present a slow, progressive and irreversible kidney function and structural damaging lasting for three or more months, at times asymptomatic. Approximately 17% of the adult population worldwide presents CKD, from whose more than 20% are over 65 years old [2,3].

Kidney dysfunction can cause systemic damage to the patient's health, which justifies its individual monitoring in order to improve the clinical outcomes [4].

Hypertension can be a cause and is often a consequence of CKD. In clinical practice, there are case of patients who cannot reduce blood pressure, explained by factors such as polypharmacy, non-adherence and pharmacokinetic disorders, specially in the metabolism and excretion [5,6]. Drugs pharmacokinetic in CKD patients is commonly altered, which can further worsened with the use of nephrotoxic drugs [7].

Among the different antihypertensives, Losartan (angiotensin II receptor antagonist) is notorious for its rapid absorption when administered orally, with peak plasma concentrations from one to two hours after administration. Its active metabolite, Acid Losartan, is up to 40 times more active [8]. Although this drug is effective and provides a safe option to patients due to the low prevalence of adverse reactions, there are controversies regarding its use in individuals with kidney disease due to the risk of nephrotoxicity [9,10].

Therapeutic drug monitoring (TDM) has become an important tool to determine effectively the plasma levels of therapeutic agents such as: antimicrobials, anticonvulsants, immunosuppressants, antihypertensives, among others [11–15] and thus optimize the pharmacological treatments, preventing invasive therapies, such as hemodialysis.

Over the years, several analytical techniques have been developed and applied in TDM, such as HPLC (High performance liquid chromatography) [11,13,16,17], mass spectrometry [18–20] and mass spectrometry in tandem mode with liquid chromatography system [6,8,13,15,21–27]. To reach higher sensitivity, selectivity and promptness, new studies are investing in Ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) allowing the identification and quantification of analytes in very low concentrations and reduced volumes [6,28–31].

Van der Nagel et al. described an UPLC-MS/MS method for quantifying 8 antihypertensives (including Losartan) and its active metabolites in human plasma, using triple quadrupole detector in multiple reaction monitoring mode, applying positive and negative electrospray ionization [6]. In this study, we used real samples from chronic kidney patients and the mass spectrometer timsTOF (Bruker) with Electrospray Source (ESI) in positive mode and a Q-TOF analyzer (Ultra-High Resolution Qq-Time-Of-Flight), promoting greater sensitivity, speed, high resolution and reliability for analysis [32,33].

The present paper aimed to develop and validate a UHPLC-MS/MS method for simultaneous quantification of the antihypertensive Losartan and Losartan Acid (EXP3174) in plasma samples from CKD patients.

2. Material and methods

2.1. Sample collection

CKD patients from Alberto Lima Hospital in the city of Macapá, Brazil were selected according to the following inclusion criteria: hypertension and chronic use of Losartan. The plasma collection was set according to the pharmacokinetic profile of Losartan [34–36]. Two blood samples of 5 ml each were collected, one before the maintenance dose and another

after 1 hour and 15 minutes after administration. The samples were immediately centrifuged (at 3000 rpm for 10 min) to separate the plasma fraction, which was stored in a freezer at -40° C until the analysis. The experimental protocol was in accordance with the ethical standards of the Research ethics committees of the Federal University of Amapá (CAAE nº 76695717.0.0000.0003).

2.2. Chemicals and reagentes

The analytical standards of Losartan, Losartan Carboxylic Acid (EXP3174) and Irbesartan (Internal Standard) were purchased from Synfine Research, Inc. (Canada). Acetonitrile HPLC gradient (≥99.9%) grade and Methanol were obtained from Labbox Labware S.L (Spain) and Acetonitrile HPLC supra gradient grade and Formic acid were obtained from Scharlab S.L (Spain).

2.3. Equipment and analytic conditions

An Ultra-high performance liquid chromatography systems (Elute LC series from Bruker®) coupled to a high-resolution mass spectrometer (timsTOF from Bruker®) has been used for the method validation, using an Electrospray Source (ESI) in positive mode and a Q-TOF analyzer (Ultra-High Resolution Qq-Time-Of-Flight), according to the conditions listed in **Table 1.** The method was developed based on previous studies [23].

Table 1.

Gradient elution timetable and mass spectrometer parameters

Time (min)	**%A**	**%B**
0.0	95.0	5.0
0.40	95.0	5.0
0.50	65.0	35.0
7.00	0.0	100.0
12.00	0.0	100.0
12.10	95.0	5.0
15.00	95.0	5.0
Column Oven (°C)	35 °C	
Injection Volume	2 µl	
Column: Intensity Solo	C18, 100 x 2.1 mm; 2µm	
Parameters	**Value**	
Ionization source	ESI	
Ion Polarity	Positive	
Scans	50-1000 m/z	
Nebulizer	2.5Bar	
Dry gas	6.0 l/min	
Dry Heater	200 °C	
Capillary voltage	4500 V	

A: Ultrapure water containing 0.1% (v/v) formic acid;

B: Methanol containing 0.1% (v/v) formic acid

The validation was divided in two complementary stages, the first referring to the analytical profile of the analytes in organic solvent, while the second stage, bioanalytical validation, focused on the analytes responses in different biological matrixes.

2.4. Stock Solutions and Quality Control Samples

The stock solutions of Losartan (500 µg/mL), Losartan Acid (370 µg/mL) and the internal standard Irbesartan (500 µg/mL) were prepared in acetonitrile (Labbox®).

Working solutions were obtained by diluting the stock solutions to reach the concentrations required for validation, providing a concentration range of 0.002 – 0.5 µg/mL for Losartan and 0.005 – 1.5 µg/mL for Losartan Acid.

Six quality control samples were prepared from the working solutions at concentrations of 1.5, 0.5 and 0.025 µg/mL for Losartan and 2.775, 0.925 and 0.288 µg/mL for Losartan Acid.

2.5. Preparation of spiked samples

To assess the real scenario, an amount of blank human plasma (300 µL) was spiked with the working solutions containing Losartan, Losartan Acid and Irbesartan, to reach a concentration range of 0.005 - 1 µg/mL for Losartan and 0.001 - 6 µg/mL for Losartan Acid for calibration curve and quality controls at the concentrations listed in 2.4. After that, 300µL of acetonitrile HPLC gradient grade was added to each aliquot, vortex stirred and immediately centrifuged (at 3000 rpm for 10 min). The supernatant was filtered on a syringe filter (GHP 0.2 µm Acrodisc®) and injected into the chromatographic system.

2.6. Plasma samples of kidney patients

A plasma aliquot of 300µL from each CKD patient was spiked solely with the internal standard working solution. After that, acetonitrile HPLC gradient grade was added to reach an equal volume, vortex stirred and subsequently centrifuged (at 3000 rpm for 10 min) and the supernatant filtered on a syringe filter (GHP 0.2 µm Acrodisc®) and injected into the chromatographic system.

2.7. Method validation

The method developed was validated in accordance with the ICH Q2(R1) guidelines [37] for analytical and bioanalytical applications. The parameters Linearity, Precision (Repeatability), Accuracy, Limits of Quantification and Detection, Robustness, Selectivity and Recovery were evaluated for both analytes in acetonitrile (analytical validation) and spiked human plasma (bioanalytical validation) to fully validate the proposed method for TDM of losartan and losartan acid.

2.7.1. Linearity, Precision (Repeatability) and Accuracy

Linearity was determined based on the analyte concentration *versus* peak area ratio, according to the calibration concentration range established for Losartan and Acid Losartan in acetonitrile (analytical validation) and human plasma (bioanalytical validation). The quality control samples prepared with the working solutions were used for the precision and accuracy assay, expressed as mean ± coefficient of variation (% CV) and percentage ± standard deviation (SD), respectively.

2.7.2. Limits of Quantification and Detection

The limits of quantification and detection were determined from the standard deviation of the response and the slope of the analytical curve, as established in the guideline. The estimation of the limit of detection (LoD) and limit of quantification (LoQ) can be made based on the calculation from the signal-to-noise ratio (LoD and LoQ correspond to 3 and 10 times the noise level, respectively).

2.7.3. Robustness

The method robustness was evaluated by altering two different parameters: extractive solvent (using Acetronitrile HPLC supra gradient grade) and lack of stirring during the sample preparation. The results were expressed as percent change (%).

2.7.4. Selectivity

The method selectivity was assessed by assuring that the endogenous substances present in human plasma do not interfere in the analytes quantification. Samples of normal, lipemic and hemolysate plasma from six healthy volunteers were used to verify the selectivity of the bioanalytical method. The results were also expressed in terms of percent change (%).

2.7.5. Recovery

Quality control samples and their respective concentrations measured in analytical and bioanalytical validation were used to quantify the recovery for both analytes. Quality control samples prepared in acetonitrile HPLC gradient grade (non-extracted samples) accounted for 100% recovery, while plasma quality control samples (extracted samples) had their respective recovery percentages calculated.

3. Results and discussion

3.1. Method validation

The analytical validation of the UHPLC-MS/MS method prepared the technique to receive the plasma samples, whose calibration curves, within the established concentration ranges (item 2.4), were linear for both analytes, with a determination coefficient higher than 0.99. According to the data presented in **Table 2** and **Table 3**, the method was precise, with CV values ranging between 0.50 and 4.89%, accurate as these values varied between 88.85 – 102.69%. The low detection and quantification limits of Losartan (LoD 0.002 µg/mL, LoQ 0.005 µg/mL) and Losartan Acid (LoD 0.004 µg/mL, LoQ 0.01 µg/mL) confirmed the high sensitivity of the method developed. Additionally, the method was robust with the change on the acetonitrile purity and lack of stirring (2.7.3), presenting low variations in the analytical measurement

The bioanalytical validation confirmed the effectiveness of the analytical performance. The calibration curves developed in the bioanalytical phase presented a strong linear relationship, with the R^2 value higher than 0.99. The method also reached the minimum parameters of precision and accuracy, with CV values ranging between 0.52 and 9.20% and accuracy with values ranging from 85.07 – 113.51% thus demonstrating the agreement between the plasma spiked concentrations and the value measured by the developed method. The limits of detection and quantification of Losartan (LoD 0.0005µg/mL, LoQ 0.005 µg/mL) and Losartan Acid (LoD 0.002 µg/mL, LoQ 0.005 µg/mL) remained very low, confirming the high sensitivity of the method in human plasma. Robustness in the bioanalytical stage, also presented low variations in the analytical measurements, confirming that even in plasma matrix, the method was able to withstand the proposed changes. The data in **Table 2** and **Table 3** show the selectivity of the method for the

quantification of analytes in different human plasma (since the measurements in the different matrices varied from -6.95 to 11.348). The extent of method selectivity will imply laboratory routine, preventing lipid or hemolysate samples become analytical interferers, since such variations don't preclude precise and accurate quantification of the analyte for this validated method. The obtained recovery (98.06 – 111.02%), comparing between non-extracted and extracted samples has shown the extraction method effectiveness. Despite Losartan and its metabolite have a plasma protein binding rate ≥ 99% [34–36,38], the method was able to efficiently extract both analytes.

Table 2.

Validation results of UHPLC–MS/MS method for determination of Losartan in Acetonitrile HPLC gradient grade and human plasma.

Losartan	Analytical method validation		Bioanalytical method validation		
Validation parameters					
Linearity (n = 3)		R^2: 1 y = 2*107x – 9021.5		R^2: 1 y = 2*107x + 3836.3	
Sensitivity, µg/mL		LoQ: 0.005 LoD: 0.002		LoQ: 0.005 LoD: 0.0005	
		% ± SD		% ± SD	
Accuracy (n = 9), µg/mL	[1.51]	92.8 ± 0.05	[1.51]	101.67 ± 0.14	
	[0.75]	102.69 ± 0.03	[0.75]	113.51 ± 0.01	
	[0.025]	99.4 ± 0.0007	[0.025]	110.37 ± 0.002	
		Mean ± %CV		Mean ± %CV	
Precision (n = 9), µg/mL	[1.51]	1.39 ± 4.22	[1.51]	1.52 ± 9.20	
	[0.75]	0.770 ± 4.89	[0.75]	0.85 ± 1.43	
	[0.025]	0.025 ± 3.08	[0.025]	0.027 ± 7.37	
		%Change		%Change	
Robustness (n = 3), µg/mL	[0.75][a]	-5.26	[0.75[a]]	10.53	
	[0.75][b]	-14.57	[0.75[b]]	12.30	
				Mean ± SD	% Change
Selectivity		unvalued	[0.75[1]]	0.77 ± 0.03	2.74
			[0.75[2]]	0.79 ± 0.94	5.99
			[0.75[3]]	0.83 ± 0.04	11.348
				%	
Recovery		unvalued	[1.51]	109.55	
			[0.75]	110.53	
			[0.025]	111.02	

[1]Normal plasma, [2]Lipemic plasma, [3]Hemolysate plasma; [a]Acetronitrila HPLC supra gradient grade, [b]non-agitated.

Table 3.

Validation results of UHPLC–MS/MS method for determination of EXP3174 in Acetonitrile HPLC gradient grade and human plasma.

EXP3174	Analytical method validation		Bioanalytical method validation		
Validation parameters					
Linearity (n = 3)		R^2: 0.9966 $y = 1*10^7x - 257159$		R^2: 0.9974 $y = 7*10^6x - 88956$	
Sensitivity, µg/mL		LoQ: 0.01 LoD: 0.004		LoQ: 0.005 LoD: 0.002	
		% ± SD		% ± SD	
Accuracy (n = 9), µg/mL	[2.775]	91.58 ± 0.04	[2.775]	90.20 ± 0.16	
	[0.925]	89.42 ± 0.02	[0.925]	87.69 ± 0.004	
	[0.278]	88.85 ± 0.001	[0.278]	85.07 ± 0.005	
		Mean ± %CV		Mean ± %CV	
Precision (n = 9), µg/mL	[2.775]	2.541 ± 1.59	[2.775]	2.503 ± 6.60	
	[0.925]	0.827 ± 3.29	[0.925]	0.811 ± 0.52	
	[0.278]	0.247 ± 0.50	[0.278]	0.236 ± 2.29	
		%Change		%Change	
Robustness (n = 3), µg/mL	[0.925[a]]	-6,80	[0.925[a]]	-5,18	
	[0.925[b]]	-10,8	[0.925[b]]	13	
				Mean ± SD	% Change
Selectivity		unvalued	[0.75[1]]	0.860 ± 0.09	-6.95
			[0.75[2]]	0.941 ± 0.23	1.74
			[0.75[3]]	0.908 ± 0.02	-1.77
				%	
Recovery		unvalued	[2.775]	98.49	
			[0.925]	98.06	
			[0.278]	95.74	

[1]Normal plasma, [2]Lipemic plasma, [3]Hemolysate plasma; [a]Acetronitrila HPLC supra gradient grade, [b]non-agitated.

3.2. Patient samples quantification

Five volunteers attended for blood collection. Losartan (50 or 100mg) was administered orally prior to blood sampling. Figure 1 shows Losartan and its metabolite plasmatic levels found before and after administration to patients. Forty percent of the patients presented subtherapeutic levels of Losartan and Losartan acid after administration. This circumstance may result in poor blood pressure control since the drug and the metabolite did not reach the desired therapeutic levels. The data also suggest problems in the metabolism of Losartan, we can observe that patient 3 (P3a), after administration of Losartan, has plasma concentrations very close to the minimum toxic concentration,

however when the metabolite is analyzed, it is noted that the concentrations are below the minimum effective concentration, suggesting that losartan is not being completely metabolized (Fig. 1). Therefore, the need to monitor this antihypertensive drug administered to renal patients is reinforced either by the inadequate blood pressure control as well as to the risk of kidney disease progression.

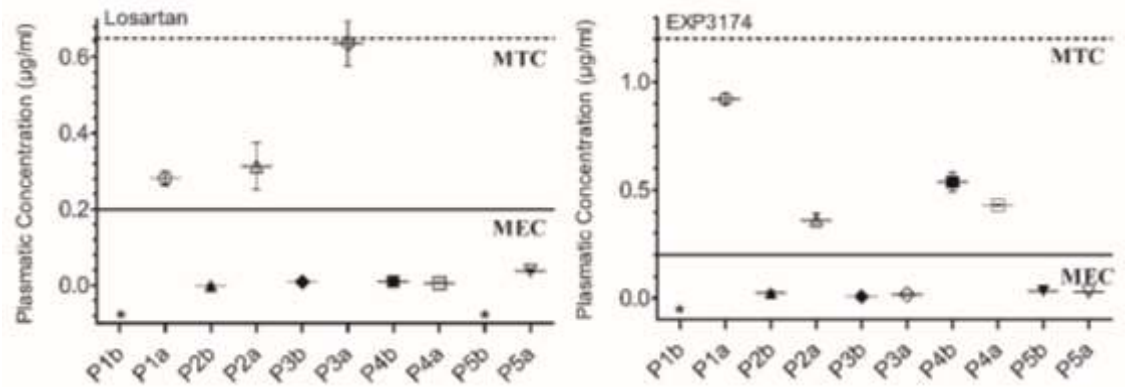

Fig. 1. Mean concentrations of Losartan and Carboxylic Acid Losartan (EXP3174) determined by UHPLC-MS/MS method in plasma samples of five volunteers (n = 5). Patients P1, P2, P3 have taken Losartan 50 mg, while patients P4 and P5 have administered Losartan 100 mg. No analytes detected (*), before to administration (b), after administration (a), minimum effective concentration (MEC), minimum toxic concentration (MTC) [39].

4. Conclusion

The UHPLC-MS/MS method proposed and validated in the present study is suitable for the efficient and rapid quantification of Losartan and Carboxylic Acid Losartan in human plasma. The validation parameters highlighted a linear, sensitive, selective method with excellent extraction efficiency and all data according to the bioanalytical requirements, enabling its application in the clinical routine.

Based on the validation and applicability given, Losartan and its active metabolite could become new candidates for therapeutic monitoring in kidney patients. The methodology developed creates possibilities for clinical protocols contributing to improve the hypertension treatment of CKD patients.

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References

[1] U.D. de A. Silva, Avaliação dos riscos associados à farmacoterapia e impacto do seguimento farmacoterapêutico de pacientes renais crônicos do Estado do Amapá, Universidade Federal do Amapá, 2017. http://www2.unifap.br/ppgcf.

[2] Y. Gagnebin, J. Boccard, B. Ponte, S. Rudaz, Metabolomics in chronic kidney disease : Strategies for extended metabolome coverage, J. Pharm. Biomed. Anal. 161 (2018) 313–325. doi:10.1016/j.jpba.2018.08.046.

[3] M. Cañadas-garre, K. Anderson, J. Mcgoldrick, A.P. Maxwell, A.J. Mcknight, Proteomic and metabolomic approaches in the search for biomarkers in chronic kidney disease, J. Proteomics. in press (2018). doi:10.1016/j.jprot.2018.09.020.

[4] D.U. Silverthorn, Fisiologia Humana: uma abordagem integrada, fifth ed., Artmed, Porto Alegre, 2010.

[5] A.B. Marquito, N.M.S. Fernandes, F.A.B. Colugnati, R.B. Paula, Interações medicamentosas potenciais em pacientes com doença renal crônica, J Bras Nefrol. 36 (2014) 26–34. doi:10.5935/0101-2800.20140006.

[6] B.C.H. van der Nagel, J. Versmissen, S. Bahmany, T. van Gelder, B.C.P. Koch, High-throughput quantification of 8 antihypertensive drugs and active metabolites in human plasma using UPLC–MS/MS, J. Chromatogr. B. 1060 (2017) 367–373. doi:10.1016/j.jchromb.2017.06.029.

[7] J.I.S. Russo, Nefrotoxicidade induzida por fármacos: Caracterização da realidade hospitalar, medidas preventivas e oportunidades de intervenção, Universidade de Lisboa, 2013. http://hdl.handle.net/10451/11257.

[8] V. Babarahimi, Z. Talebpour, F. Haghighi, N. Adib, H. Vahidi, Validated determination of losartan and valsartan in human plasma by stir bar sorptive extraction based on acrylate monolithic polymer, liquid chromatographic analysis and experimental design methodology, J. Pharm. Biomed. Anal. 153 (2018) 204–213. doi:10.1016/j.jpba.2018.02.030.

[9] P. Dionisio, M. Valenti, E. Caramello, R. Bergia, R. Cravero, I.M. Berto, B. Agostini, G. Monaci, P. Bajardi, [Acute kidney failure and losartan: a recently observed event of antagonists of angiotensin II AT1 receptors], Minerva Urol Nefrol. 52 (2000) 123–125. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11227361.

[10] R. Rastghalam, M. Nematbakhsh, M. Bahadorani, F. Eshraghi-Jazi, A. Talebi, M. Moeini, F. Ashrafi, S. Shirdavani, Angiotensin Type-1 Receptor Blockade May Not Protect Kidney against Cisplatin-Induced Nephrotoxicity in Rats, ISRN Nephrol. 2014 (2014) 1–7. doi:10.1155/2014/479645.

[11] J. Weber, S. Oberfeld, A. Bonse, K. Telger, R. Lingg, G. Hempel, Validation of a dried blood spot method for therapeutic drug monitoring of citalopram, mirtazapine and

risperidone and its active metabolite 9-hydroxyrisperidone using HPLC–MS, J. Pharm. Biomed. Anal. 140 (2017) 347–354. doi:10.1016/j.jpba.2017.02.061.

[12] D.J. Touw, C. Neef, A.H. Thomson, A.A. Vinks, Cost-effectiveness of therapeutic drug monitoring: A systematic review, Ther. Drug Monit. 27 (2005) 10–17. doi:10.1097/00007691-200502000-00004.

[13] K.M. Matar, Quantification of levetiracetam in human plasma by liquid chromatography-tandem mass spectrometry: Application to therapeutic drug monitoring, J. Pharm. Biomed. Anal. 48 (2008) 822–828. doi:10.1016/j.jpba.2008.05.035.

[14] A.S. Machado, O impacto do monitoramento terapêutico de antimicrobianos sobre o tratamento e mortalidade intra-hospitalar de pacientes em uma UTI de queimados, Universidade de São Paulo, 2016. http://www.teses.usp.br/teses/disponiveis/5/5134/tde-04112016-152417/es.php.

[15] C. Liao, S. Chang, S. Hu, Z. Tang, G. Fu, Rapid and sensitive liquid chromatography-tandem mass spectrometry method for determination of 1-β-d-arabinofuranosyluracil in human plasma and application to therapeutic drug monitoring in patient with leukemia, J. Pharm. Biomed. Anal. 85 (2013) 118–122. doi:10.1016/j.jpba.2013.07.015.

[16] S. Brinker, A. Pandey, C. Ayers, A. Price, P. Raheja, D. Arbique, S.R. Das, E.A. Halm, N.M. Kaplan, W. Vongpatanasin, Therapeutic Drug Monitoring Facilitates Blood Pressure Control in Resistant Hypertension, J. Am. Coll. Cardiol. 63 (2014) 834–835. doi:10.1016/j.jacc.2013.10.067.

[17] W. Reis do Carmo, F.F. Ferreira, R. Diniz, Phase quantification of antihypertensive drugs – Chlorthalidone, Hydrochlorothiazide, Losartan and combinations, Losartan/Chlorthalidone and Losartan/Hydrochlorothiazide – by the Rietveld method, J. Pharm. Biomed. Anal. 88 (2014) 152–156. doi:10.1016/j.jpba.2013.08.035.

[18] S. D'Aronco, E. D'Angelo, S. Crotti, P. Traldi, M. Agostini, New Mass Spectrometric Approaches for the Quantitative Evaluation of Anticancer Drug Levels in Treated Patients, Ther. Drug Monit. 41 (2019) 1–10. doi:10.1097/FTD.0000000000000573.

[19] H.H. Maurer, Mass spectrometry for research and application in therapeutic drug monitoring or clinical and forensic toxicology, Ther. Drug Monit. 40 (2018) 389–393. doi:10.1097/FTD.0000000000000525.

[20] P.B. Ryan, R.E. Hunter, S.A. Radford, D.B. Barr, J.R. Cohen, X. Chen, P. Panuwet, P.E. D'Souza, K. Kartavenka, M.E. Marder, Biological Matrix Effects in Quantitative Tandem Mass Spectrometry-Based Analytical Methods: Advancing Biomonitoring, Crit. Rev. Anal. Chem. 46 (2015) 93–105. doi:10.1080/10408347.2014.980775.

[21] V.K. Karra, N.R. Pilli, J.K. Inamadugu, J.V.L.N.S. Rao, Simultaneous determination of losartan, losartan acid and amlodipine in human plasma by LC-MS/MS and its application to a human pharmacokinetic study., Pharm. Methods. 3 (2012) 18–25. doi:10.4103/2229-4708.97711.

[22] V.R. Acquaro Junior, Development of different LC-MS/MS methods for the determination of drugs and endocannabinoids in plasma samples, Universidade de São

Paulo, 2018. http://www.teses.usp.br/teses/disponiveis/59/59138/tde-31052018-223216/pt-br.php.

[23] B. Prasaja, L. Sasongko, Y. Harahap, Hardiyanti, W. Lusthom, M. Grigg, Simultaneous quantification of losartan and active metabolite in human plasma by liquid chromatography-tandem mass spectrometry using irbesartan as internal standard, J. Pharm. Biomed. Anal. 49 (2009) 862–867. doi:10.1016/j.jpba.2009.01.007.

[24] M.C. Salvadori, R.F. Moreira, B.C. Borges, M.H. Andraus, C.P. Azevedo, R.A. Moreno, N.C. Borges, Simultaneous determination of losartan and hydrochlorothiazide in human plasma by LCMSMS with electrospray ionization and its application to pharmacokinetics, Clin. Exp. Hypertens. 31 (2009) 415–427. doi:10.1080/10641960802668714.

[25] F. Aucella, V. Lauriola, G. Vecchione, G.L. Tiscia, E. Grandone, Liquid chromatography-tandem mass spectrometry method as the golden standard for therapeutic drug monitoring in renal transplant, J. Pharm. Biomed. Anal. 86 (2013) 123–126. doi:10.1016/j.jpba.2013.08.001.

[26] M. Polinko, K. Riffel, H. Song, M.W. Lo, Simultaneous determination of losartan and EXP3174 in human plasma and urine utilizing liquid chromatography/tandem mass spectrometry, J. Pharm. Biomed. Anal. 33 (2003) 73–84. doi:10.1016/S0731-7085(03)00348-0.

[27] A. Kumar Pandey, R. Rapolu, C.K. Raju, G. Sasalamari, S. Kumar Goud, A. Awasthi, S.G. Navalgund, K. V. Surendranath, The novel acid degradation products of losartan: Isolation and characterization using Q-TOF, 2D-NMR and FTIR, J. Pharm. Biomed. Anal. 120 (2016) 65–71. doi:10.1016/j.jpba.2015.11.037.

[28] M. Simiele, A. Ariaudo, A. De Nicolò, F. Favata, M. Ferrante, C. Carcieri, S. Bonora, G. Di Perri, A. D'Avolio, UPLC–MS/MS method for the simultaneous quantification of three new antiretroviral drugs, dolutegravir, elvitegravir and rilpivirine, and other thirteen antiretroviral agents plus cobicistat and ritonavir boosters in human plasma, J. Pharm. Biomed. Anal. 138 (2017) 223–230. doi:10.1016/j.jpba.2017.02.002.

[29] M.N. Paludetto, F. Puisset, F. Le Louedec, B. Allal, T. Lafont, E. Chatelut, C. Arellano, Simultaneous monitoring of pazopanib and its metabolites by UPLC–MS/MS, J. Pharm. Biomed. Anal. 154 (2018) 373–383. doi:10.1016/j.jpba.2018.03.013.

[30] A. De Nicolò, V. Avataneo, F. Rabbia, G. Bonifacio, J. Cusato, C. Tomasello, E. Perlo, P. Mulatero, F. Veglio, G. Di Perri, A. D'Avolio, UHPLC–MS/MS method with protein precipitation extraction for the simultaneous quantification of ten antihypertensive drugs in human plasma from resistant hypertensive patients, J. Pharm. Biomed. Anal. 129 (2016) 535–541. doi:10.1016/j.jpba.2016.07.049.

[31] P.O.M. Gundersen, A. Helland, O. Spigset, S. Hegstad, Quantitation of 21 antihypertensive drugs in serum using UHPLC-MS/MS, J. Chromatogr. B. (2018). doi:10.1016/j.jchromb.2018.04.038.

[32] M.A. Park, timsTOF MS for Life Science Problem Solving, Genet. Eng. Biotechnol. News. 36 (2016) 16–17. doi:10.1089/gen.36.19.08.

[33] V. D'Atri, T. Causon, O. Hernandez-Alba, A. Mutabazi, J. Veuthey, S. Cianferani, D. Guillarme, Adding a new separation dimension to MS and LC-MS: What is the utility of ion mobility spectrometry?, J. Sep. Sci. 41 (2018) 20–67. doi:10.1002/jssc.201700919.

[34] D.A. Sica, T.W.B. Gehr, S. Ghosh, Clinical pharmacokinetics of losartan, Clin. Pharmacokinet. 44 (2005) 797–814. doi:10.2165/00003088-200544080-00003.

[35] A.-R.A. Al-Majed, E. Assiri, N.Y. Khalil, H.A. Abdel-Aziz, Losartan: Comprehensive Profile, in: Profiles Drug Subst. Excipients Relat. Methodol., 1st ed., Elsevier Inc., 2015: pp. 159–194. doi:10.1016/bs.podrm.2015.02.003.

[36] M.W. Lo, M.R. Goldberg, J.B. McCrea, H. Lu, C.I. Furtek, T.D. Bjornsson, Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans, Clin. Pharmacol. Ther. 58 (1995) 641–649. doi:10.1016/0009-9236(95)90020-9.

[37] ICH Expert Working Group, Validation of analytical procedures: text and methodology Q2 (R1), Geneva, 2005. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products.

[38] G.D.O.R. Fernandes, D.R. Fernandes, R.D. Cavalcante Filho, L.F. De Pontes, A.T. Terra Júnior, Efeitos farmacológicos decorrentes ao bloqueio dos receptores AT1, Rev. Científica FAEMA. 8 (2017) 139. doi:10.31072/rcf.v8i2.588.

[39] M. Schulz, A. Schmoldt, Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics, Pharmazie. 58 (2003) 447–474. doi:10.1186/cc11441.

5 CONSIDERAÇÕES FINAIS

O método desenvolvido por CLUE-EM/EM nesse estudo, foi adequado para quantificação de Losartana e Ácido Carboxilico Losartana em plasma humano, sendo considerado simples, rápido e de baixo custo. Os parâmetros de validação destacaram um método linear, sensível, repetível, exato, robusto, seletivo e com excelente eficiente de extração, respeitando a regulamentação vigente.

A metodologia desenvolvida cria possibilidades para implantação de protocolos clínicos que visem o monitoramento terapêutico de Losartana, otimizando o tratamento farmacológico de pacientes renais, trazendo eficiência e segurança no tratamento do mesmo. É possível fazer do monitoramento deste fármaco parte da rotina dos laboratórios de análises clínicas, uma vez que a coleta de sangue é uma pratica protocolada, segura e de fácil processamento da matriz.

A partir dos achados, foi possível demonstrar a importância do monitoramento terapêutico na condução das terapias farmacológicas para os pacientes renais crônicos, de modo a melhorar a qualidade de vida dos pacientes e reduzir os gastos no meio clinico.

REFERÊNCIAS

AL-MAJED, A.-R. A. et al. Losartan: Comprehensive Profile. In: **Profiles of Drug Substances, Excipients and Related Methodology**. 1. ed. [s.l.] Elsevier Inc., 2015. 40p. 159–194.

CAÑADAS-GARRE, M. et al. Proteomic and metabolomic approaches in the search for biomarkers in chronic kidney disease. **Journal of Proteomics**, v. in press, 2018.

COUCHMAN, L.; FISHER, D. S.; SUBRAMANIAM, K.; HANDLEY, S. A.; BOUGHTFLOWER, R. J.; BENTON, C. M.; FLANAGAN, R. J. Ultra-fast LC-MS/MS in therapeutic drug monitoring: quantification of clozapine and norclozapine in human plasma. **Drug Testing and Analysis**, p. 1-7, 2017.

DA SILVA, D. F. M. D. **Monitorização Terapêutica de Fármacos: relevância clínica e forense**. Dissertação (Mestrado integrado em Medicina) – Faculdade de Medicina, Universidade de Porto. Porto, p.44, 2014.

GAGNEBIN, Y. et al. Metabolomics in chronic kidney disease : Strategies for extended metabolome coverage. **Journal of Pharmaceutical and Biomedical Analysis**, v. 161, p. 313–325, 2018.

GILS, A. Combining Therapeutic Drug Monitoring with Biosimilars, a Strategy to Improve the Efficacy of Biologicals for Treating Inflammatory Bowel Diseases at an Affordable Cost. **Digestive Diseases**, v. 35, n. 1–2, p. 61–68, 2017.

LESOSKY, M.; JOSKA, J.; DECLOEDT, E. Simulating therapeutic drug monitoring results for dose individualisation to maintain investigator blinding in a randomised controlled trial. **Trials**, v. 18, n. 1, p. 261, 2017.

LO, M. W. et al. Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans. **Clinical Pharmacology and Therapeutics**, v. 58, n. 6, p. 641–649, 1995.

RICHARDS, P. G.; DANG, K. M.; KAUFFMAN, C. A.; STALKER, K. L.; SUDEKUM, D.; KERR, L.; BRINKER-BODLEY, M.; CHERIYAN, B.; WEST, N.; COLLINS, C. D.; POLEGA, S.; MALANI, A. N. Therapeutic drug monitoring and use of an adjusted body weight strategy for high-dose voriconazole therapy. **Journal of Antimicrobial Chemotherapy**, n. 72, p. 1178–1183, 2017.

SCHMITZ, E. M. H.; BENOY - DE KEUSTER, S.; MEIER, A. J. L.; SCHARNHORST, V.; TRAKSEL, R. A. M.; BROEREN, M. A. C.; DERIJKS, L. J. J. Therapeutic drug monitoring (TDM) as a tool in the switch from infliximab innovator to biosimilar in rheumatic patients: results of a 12-month observational prospective cohort study. **Clinical Rheumatology**, p. 1–6, 2017.

SILVA, U. D. A. **Avaliação dos riscos associados a medicamentos e o impacto do seguimento farmacoterapêutico em pacientes renais do estado do amapá**. Dissertação (Mestrado) – Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Amapá. Macapá, p.75, 2017.

SILVERTHORN, D. U. **Fisiologia Humana**: uma abordagem integrada. 5 ed. Porto Alegre: Artmed. 2010.

STOTT, K. E.; HOPE, W. W. Therapeutic drug monitoring for invasive mould infections and disease: pharmacokinetic and pharmacodynamic considerations. **Journal of Antimicrobial Chemotherapy**, v. 72, n. suppl_1, p. i12–i18, 2017.

TOUW, D. J. et al. Cost-effectiveness of therapeutic drug monitoring: A systematic review. **Therapeutic Drug Monitoring**, v. 27, n. 1, p. 10–17, 2005.

Anexo 1 – Parecer do Comitê de Ética

UNIVERSIDADE FEDERAL DO AMAPÁ
COMITÊ DE ÉTICA EM PESQUISA

CERTIFICADO

Título da Pesquisa: Implementação e avaliação do impacto do monitoramento plasmático de fármacos em pacientes renais.
Pesquisador Responsável: ALBERTO GOMES TAVARES JUNIOR
CAAE: 76695717.0.0000.0003
Submetido em: 11/09/2017
Instituição Proponente: Pró-Reitoria de Pesquisa e Pós-Graduação

Número do Parecer: 2.301.285

Certificamos que o Projeto cadastrado está de acordo com os Princípios Éticos na Experimentação Humana, adotados pelo Comitê Nacional de Ética em Pesquisa – CONEP, e foi aprovado pelo Comitê de Ética em Pesquisa (CEP) da Universidade Federal do Amapá (UNIFAP), em reunião realizada em 27/09/2017.

Macapá, 27 de setembro de 2017

Raphaelle Souza Borges
Comitê de Ética em Pesquisa
Portaria 051/2015

Prof.ª. Msc. Raphaelle Sousa Borges
Coordenadora - CEP-UNIFAP
Coordenadora do Comitê de Ética em Pesquisa/PROPESPG
Portaria nº 051/2015

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Anexo 2 – Normas de publicação dos respectivos periódicos

Brazilian Journal of Nephrology

BRIEF COMMUNICATION
A brief communication is a report on a single subject, which should be concise but conclusive. Like original articles, these papers should present unpublished material, but that have less significance and of particular interest in the area of nephrology, presenting preliminary results of immediate relevance.

The manuscript should contain:

- A structured abstract (Introduction, Methods, Results, and Conclusion), with up to 250 words;
- No more than 7 key words;
- The text should be divided in Introduction, Methods, Results, Discussion, with up to 1500 words;
- No more than 15 references;
- No more than three illustrations (figures and/or tables).

a. Structure and Preparation of Manuscripts

The main document should be sent in a word file (.doc or .rtf), double spaced, font size of 12, margin of 3 cm on each side, pages numbered in Arabic numerals, and each section should start in a new page, consecutively: a) title page; b) summary and key words; c) text body; d) acknowledgments; e) references; f) tables and subtitles (excluding images, which must be sent separately in .jpg or .tiff format).

b. **Title Page**

- **Title of the manuscript:** should be concise and complete, describing the subject to which it refers (superfluous words should be omitted). For manuscripts submitted in Portuguese, an English version of the title must be included;
- **Running title** of the manuscript that must correspond to the Portuguese and/or English version of the title;
- **Names of the authors**, indicating the respective academic degree;
- **Authors' affiliations** with the hierarchical units presented in descending order (university, school, and department). The names of the institutions should be presented in full in the institution's original language or in the English version when Latin words are not used. Affiliations should not be accompanied by the authors' titles or mini-CVs. All authors must provide an ORCID ID (Open Researcher and Contributor ID, http://orcid.org/) at the time of submission by entering it in the user profile in the submission system;
- **Corresponding author**, with the respective e-mail;
- **Name of the funding agency** of the study;
- **Title, year, and institution of submission**, for manuscripts based on an academic thesis;
- **Name of the event, location and date of presentation**, for manuscripts based on a presentation at a scientific meeting;
- **Declaration of conflict of interest;**
- **Indication of authors' contribution.**

c. **Abstract and key words**

- **Abstract**: including introduction, procedures and conclusions of the study (maximum of 250 words). Structured abstracts should present, at the beginning of each paragraph, the subsections names (Introduction, Method, Results and Discussion);

- **Key words**: ewords that represent the subject of the study, should be presented in numbers of 3 to 7, supplied by the author, based on DECS - Descritores em Ciências da Saúde (http://decs.bvs.br/) or MeSH - Medical Subject Headings (http://www.ncbi.nlm.nih.gov/mesh).

d. **Text**

The main text must obey the required structure for each article category (See Types of Articles). Citations and references cited in the legends of table and figures should be numbered consecutively in the order that they appear in the text (numerical index). The references should be cited in the text with a superscript number and without parentheses as in the following example (References[1]).

Figures (photographs, graphs, drawings, etc.) should be sent individually in JPG or TIFF format (in high resolution - 300 dpi) and can be colored. They should be numbered consecutively with Arabic numerals in the order in which they were cited in the text and sufficiently clear to permit their reproduction. Figure captions should be given together with the tables, after the references. Photocopies are not accepted. For figures from previously published works, authors must provide permission in writing for their reproduction. This authorization must accompany the manuscripts submitted for publication.

Other aspects to consider:

- **Statistical analysis**: he authors should demonstrate that the statistical procedures were appropriate to test the hypothesis of the study, and that the results were correctly interpreted. The levels of statistical significance (e.g., $p < 0,05$; $p < 0,01$; $p < 0,001$) should be reported.

- **Abbreviations**: should be indicated in the text upon the first use. Thereafter, the full name should not be repeated.

- **Name of medication**: the generic name should be used.

- **Citation of machines and equipment**: all machines and equipment cited should include the model and name, state and country of manufacturer.

e. **Acknowledgments**

Acknowledgments should include all people, groups or institutions that deserve recognition, but are not included as authors; acknowledgment for financial support, technical assistance, etc., should appear before the references.

f. **References**

References should be numbered sequentially, in the same order that they were mentioned in the text and identified with superscript numbers. The references must comply with the standard defined by the International Committee of Medical Journal Editors - ICMJE (https://www.nlm.nih.gov/bsd/uniform_requirements.html).The title and journal name should be abbreviated according to the style presented by the Index Medicus: abbreviations of journal titles (http://www2.bg.am.poznan.pl/czasopisma/medicus.php?lang=eng). Personal communications, unpublished studies, or ongoing studies should be cited only when absolutely necessary, but should not be included in the list of references; only mentioned in the text footer.

Examples:
Articles from journals (up to six authors)

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002 Jul 25;347(4):284-7.

Articles from journals (more than six authors)

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

Articles without the name of the author

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

Entire books

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Book Chapters

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Books for which the editors (organizers) are authors

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Thesis

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Papers presented at meetings

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Journals in electronic format

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

g. **Tables and Legends**

Tables and legends must comply with the specifications defined for each article type (See Article Types). In their electronic version, tables must be presented in .doc (Microsoft Word) or .xls (Microsoft Excel) format.

Journal of Pharmaceutical and Biomedical Analysis

Full Length Research Papers: These papers should describe in detail original and important pieces of work in the fields covered by the Journal.
Peer review

This journal operates a single blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. More information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

When describing mixed solvents for chromatography, extraction or other purposes, the following convention must be adopted: solvent A-solvent B-solvent C (a:b:c, v/v/v) or (a:b:c, w/w/w) where a:b:c are the proportions (by volume or weight as appropriate) of the components A, B and C, respectively.

The method of preparation of buffers should be clearly expressed, with the pH value and molarity stated in parentheses, e.g. sodium acetate (pH 4.7; 0.1 M). For mixed solvent systems, it should be clearly stated whether the pH value quoted is the pH of the original aqueous component or the apparent pH (i.e. pH*) of the mixed solvent system. Typical examples of mobile phases employed in liquid chromatography might be:acetonitrile-sodium octylsulphate (10 mM)-sodium acetate (pH 4.7;0.1 M) (25:25:50, v/v/v), and acetonitrile-sodium octylsulphate (10 mM)-sodium acetate (0.1 M)(25:25:50, v/v/v)(pH* 4.7). Discussion of the optimisation procedure for the proposed method / assay should be given in detail.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**

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Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the

author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

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[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
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[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. 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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Apêndice 1 – TCLE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O Senhor (a) está sendo convidado (a) como voluntário (a) a participar da pesquisa "**Implementação e avaliação do impacto do monitoramento plasmático de fármacos em pacientes renais**". Neste estudo se objetiva avaliar o impacto do serviço de monitoramento plasmático de fármacos com potencial nefrotóxico em pacientes renais.

Para participar do estudo, você deverá responder a um questionário sobre critérios socioeconômicos, estado de saúde e tratamento realizado, para que possamos conhecer o perfil de utilização de medicamentos dos portadores de Doença Renal no Estado do Amapá.

Caso seja selecionado, conforme os medicamentos utilizados, você será convidado a realizar uma coleta de sangue, urina ou saliva para determinar os níveis terapêuticos, assim podemos avaliar adequação das doses dos medicamentos utilizados, em virtude da função renal.

Para evitar constrangimentos, a sua identidade será mantida em sigilo, sendo identificado por número de registro, assegurando assim, a sua privacidade. As informações obtidas só serão usadas para fins científicos sem qualquer identificação pessoal. Além disso, a coleta de sangue poderia ocasionar, dor ou desconforto durante ou logo após a coleta, o que será minimizado com a utilização de um Procedimento técnico durante a coleta por profissional capacitado.

Para participar deste estudo você não terá nenhum custo, sendo oferecido uma análise da composição corporal (Bioimpedância). Você será esclarecido (a) sobre o estudo em qualquer aspecto que desejar e estará livre para participar ou recusar-se, podendo ainda retirar seu consentimento ou interromper a participação a qualquer momento. A sua participação é voluntária e a recusa em participar não acarretará qualquer penalidade ou modificação na forma em que é atendido pelo serviço de saúde, de acordo com a resolução CNS n°466/12.

Este termo de consentimento encontra-se impresso em duas vias, sendo que uma cópia será arquivada pelo pesquisador responsável, no Laboratório de Controle de Qualidade Bromatologia e Microbiolgia do curso de Farmácia da UNIFAP e a outra será entregue a você.

Assinatura do pesquisador