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DRONEDARONA – DESENVOLVIMENTO E VALIDAÇÃO DE METODOLOGIA PARA A ANÁLISE DE COMPRIMIDOS REVESTIDOS

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil

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DRONEDARONA – DESENVOLVIMENTO E VALIDAÇÃO DE METODOLOGIA PARA A ANÁLISE DE COMPRIMIDOS REVESTIDOS

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Farmacêuticas, Área de Concentração em Controle e Avaliação de Insumos e Produtos Farmacêuticos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Farmacêuticas.**

Orientador: Prof. Dra. Clarice Madalena Bueno Rolim

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DRONEDARONA - DESENVOLVIMENTO E VALIDAÇÃO DE METODOLOGIA PARA ANÁLISE DE COMPRIMIDOS REVESTIDOS

elaborada por
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como requisito parcial para obtenção do grau de
Mestre em Ciências Farmacêuticas

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Santa Maria, 24 de agosto de 2012.

Dedico esse trabalho a minha mãe, Jacira Pedroso.

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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Farmacêuticas
Universidade Federal de Santa Maria

DRONEDARONA – DESENVOLVIMENTO E VALIDAÇÃO DE METODOLOGIA PARA ANÁLISE DE COMPRIMIDOS REVESTIDOS

AUTORA: ANA ISA PEDROSO MARCOLINO
ORIENTADORA: CLARICE MADALENA BUENO ROLIM
Data e Local da Defesa: Santa Maria, 24 de agosto de 2012.

A dronedarona é um novo agente antiarrítmico análogo à amiodarona, desenvolvido com o propósito de reduzir os efeitos adversos relacionados à amiodarona. Foi aprovado para a manutenção do ritmo cardíaco normal em pacientes com fibrilação atrial e, assim, reduzir os riscos de hospitalização. Comercialmente, encontra-se disponível na forma farmacêutica de comprimidos revestidos. Não há monografias descritas em farmacopeias ou métodos na literatura para análise de dronedarona em forma farmacêutica ou matéria-prima. No presente trabalho, foi desenvolvida metodologia para a avaliação de dronedarona em forma farmacêutica e matéria-prima. O método por cromatografia líquida em fase reversa foi realizado utilizando-se coluna Waters XBridge C18 (250 mm × 4,6 mm). A fase móvel foi composta por solução tampão pH 4,9 / acetonitrila (35:65, v/v) eluída no fluxo de 1,0 mL/min e detecção no ultravioleta em 289 nm. A separação cromatográfica foi obtida no tempo de 7,0 min, sendo linear na faixa de concentração de 5-100 μg/mL (r = 0,9999). Paralelamente, desenvolveu-se e validou-se método por espectrofotometria no ultravioleta em 289 nm utilizando metanol como diluente. Também foi desenvolvido e validado método por cromatografia eletrocinética micelar utilizando nimesulida como padrão interno. As análises foram realizadas em capilar de sílica fundida (comprimento efetivo de 40 cm e diâmetro de 50 μm), mantido a 30°C, utilizando solução eletrolítica composta de tampão borato 40 mM e SDS 50 mM, pH 9,2, com detecção no ultravioleta em 216 nm. A injeção foi realizada no modo hidrodinâmico a 50 mbar durante 7 s e voltagem constante de 28 kV foi aplicada durante as análises. A separação eletroforética foi obtida em 7,0 min, sendo linear na faixa de 25-150 μg/mL (r = 0,9995). Os procedimentos foram validados considerando-se os parâmetros especificidade, linearidade, precisão, exatidão, limite de detecção e quantificação e robustez, cujos resultados cumpriram os requisitos preconizados. Os métodos propostos foram aplicados na análise quantitativa de produtos farmacêuticos, demonstrando correlação significativa dos resultados (p > 0,05). Desenvolveu-se e validou-se método por espectrofotometria no UV utilizando tampão acetato pH 4,5 como diluente e detecção em 289 nm, o qual foi aplicado para avaliar a percentagem dissolvida dos comprimidos de dronedarona. O método de dissolução foi desenvolvido utilizando como meio 900 mL de tampão acetato pH 4,5 mantido a 37°C, aparato pá e rotação de 75 rpm.

Palavras-chave: Dronedarona. Antiarrítmico. Cromatografia líquida. Espectrofotometria no UV. Eletroforese capilar. Cromatografia eletrocinética micelar. Dissolução. Validação.

ABSTRACT

Master's Degree Dissertation
Postgraduate Program in Pharmaceutical Sciences
Federal University of Santa Maria

DRONEDARONE – DEVELOPMENT AND VALIDATION OF METHODOLOGY FOR THE ANALYSIS OF FILM-COATED TABLETS

AUTHOR: ANA ISA PEDROSO MARCOLINO
ADVISER: CLARICE MADALENA BUENO ROLIM
Presentation date: Santa Maria, August 24th 2012.

Dronedarone is a new antiarrhythmic agent and amiodarone analogue developed to reduce the toxic effects related to amiodarone. Dronedarone was approved for the maintenance of the sinus rhythmic in adult patients with atrial fibrillation and to reduce the risk of hospitalization in these patients. It is commercially available as film-coated tablets. There are no official monographs in any pharmacopoeia or analytical methods described in the literature for the analysis of dronedarone in pharmaceutical dosage form or bulk form. In the present study, an analytical methodology was developed for the analysis of dronedarone in pharmaceutical dosage form and bulk form. The reversed-phase liquid chromatography method was performed using a Waters XBridge C18 column (250 mm × 4.6 mm). The mobile phase consisted of buffer solution pH 4.9 / acetonitrile (35:65, v/v), running at a flow rate of 1.0 mL/min, using photodiode array detector set at 289 nm. The chromatographic separation was obtained within 7.0 min and it was linear in the concentration range from 5.0 to 100.0 μg/mL (r = 0.9999). The spectrophotometric method was developed and validated and dronedarone was quantified at 289 nm, using methanol as diluent. The micellar electrokinetic method was also developed and validated, using nimesulide as internal standard. The analyses were performed on a fused-silica capillary (50 μm i.d.; effective length, 40 cm), using electrolyte solution consisted of 40 mm borate buffer and 50 mM SDS at pH 9.2, with detection by photodiode array detector set at 216 nm. The injection was performed using the hydrodynamic mode at 50 mbar for 7 s and a constant voltage of 28 kV was applied during analysis. The electrophoretic separation was obtained within 7.0 min and it was linear in the concentration range from 25 to 150 μg/mL (r = 0.9995). The procedures were validated evaluating parameters such as the specificity, linearity, precision, accuracy, limits of detection and quantitation and robustness, giving results within the acceptable range. The proposed methods were applied for the analysis of the pharmaceutical product, showing significant correlation between the results (p > 0.05). The spectrophotometric method was developed and validated using acetate buffer pH 4.5 as diluent and UV detection at 289 nm, which was applied to evaluate the dissolution test. The dissolution test was developed using 900 mL of acetate buffer pH 4.5 at 37°C, as dissolution medium, and paddle as apparatus at a stirring rate of 75 rpm.

Keywords: Dronedarone. Antiarrhythmic drug. Liquid chomatography. UV spectrophotometry. Capillary electrophoresis. Dissolution test. Validation.

LISTA DE ILUSTRAÇÕES

ARTIGO 1

ARTIGO 2

ARTIGO 3

LISTA DE TABELAS

ARTIGO 1

ARTIGO 2

ARTIGO 3

LISTA DE ABREVIATURAS

ANVISA	Agência Nacional de Vigilância Sanitária
AV	Atrioventricular
CL-FR	Cromatografia líquida em fase reversa
CYP	Citocromo P450
DAD	Detector de arranjo de diodos
DPR	Desvio padrão relativo
DRO	Dronedarona
EC	Eletroforese capilar
EMA	European Medicines Agency
FA	Fibrilação atrial
FDA	U. S. Food and Drug Administration
ICH	International Conference on Harmonisation
MEKC	Cromatografia eletrocinética micelar
mg	Miligrama
min	Minutos
mL	Mililitro
mm	Milímetro
r	Coeficiente de correlação
SQR	Substância química de referência
UV	Ultravioleta
USP	Farmacopéia Americana
v	Volume
ºC	Grau Celsius
μg	Micrograma
μL	Microlitro
μm	Micrometro

SUMÁRIO

INTRODUÇÃO

1 INTRODUÇÃO

A fibrilação atrial (FA) é a arritmia cardíaca encontrada com mais frequência na prática clínica (GO et al., 2001; NATTEL, 2002), resultante de etiologia cardíaca e não cardíaca (YALTA et al., 2009). Essa arritmia é um potente fator de risco para comorbidades como o acidente vascular cerebral, além de estar associada a altos custos para o sistema de saúde. A prevalência da fibrilação atrial aumenta com a idade, sendo mais comumente encontrada em pacientes idosos (GO et al., 2001).

Muitos pacientes necessitam de uma estratégia para controle do ritmo cardíaco, para prevenir a reincidência da fibrilação atrial e melhorar a qualidade de vida (PICCINI et al., 2009).

Os fármacos antiarrítmicos formam um grupo variado de substâncias que se caracterizam por suprimir ou prevenir as alterações do ritmo cardíaco (FLÓREZ, 2002). Entretanto, em tratamentos prolongados, estes agentes podem causar efeitos adversos como pró-arritmia e toxicidade em alguns sistemas (a amiodarona causa toxicidade na tireóide, pulmões e fígado) (YALTA et al., 2009). Nesse contexto, foram investidos recursos substanciais no desenvolvimento de novos agentes antiarrítmicos que minimizassem a toxicidade mantendo a eficácia antiarrítmica, melhorando assim o tratamento dos pacientes com fibrilação atrial (SINGH et al., 2010).

A dronedarona é um novo agente antiarrítmico farmacologicamente relacionado com a amiodarona, desenvolvido para o tratamento de pacientes com fibrilação atrial e aprovada pelo U.S. Food and Drug Administration (FDA) para reduzir o risco de hospitalização cardiovascular nesses pacientes. Está disponível comercialmente na forma farmacêutica de comprimidos revestidos contendo 400 mg do fármaco, sob o nome comercial de Multaq® (Sanofi-Aventis, França).

O controle de qualidade sempre esteve presente na indústria farmacêutica, visto o alto padrão de qualidade que os produtos farmacêuticos devem possuir (WATSON, 2005). O desenvolvimento e a validação de métodos analíticos são imprescindíveis para a avaliação da qualidade dos produtos farmacêuticos, a fim de assegurar que os mesmos cumpram com a legislação vigente e contribuindo assim para a eficácia e a segurança do produto.

Encontra-se na literatura apenas um método para determinação de dronedarona, o qual é aplicado a materiais biológicos. Os compêndios oficiais não possuem monografias para a

avaliação de dronedarona, tanto em matéria-prima como em forma farmacêutica. Nesse contexto, torna-se necessário desenvolver e validar metodologias para a análise de produtos farmacêuticos contendo dronedarona, de acordo com guias nacionais e internacionais, bem como desenvolver e validar método de dissolução, para avaliação *in vitro* das propriedades biofarmacêuticas dos comprimidos revestidos.

OBJETIVOS

2 OBJETIVOS

2.1 Objetivo geral

Desenvolver e validar métodos físico-químicos para a caracterização de dronedarona em produto farmacêutico, estabelecendo procedimentos e especificações para o controle de qualidade.

2.2 Objetivos específicos

- Desenvolver e validar método por cromatografia líquida em fase reversa (CL-FR) com detecção ultravioleta (UV) para determinação quantitativa de dronedarona em forma farmacêutica;
- Desenvolver e validar método por espectrofotometria no UV para determinação quantitativa de dronedarona em forma farmacêutica;
- Desenvolver e validar método por eletroforese capilar, utilizando modo de separação por cromatografia eletrocinética micelar, para determinação quantitativa de dronedarona em forma farmacêutica;
- Realizar estudo comparativo dos métodos validados;
- Avaliar a estabilidade do fármaco através de um estudo preliminar de degradação forçada, sob condições de estresse relevantes: luz, calor, umidade, hidrólise ácida, hidrólise básica e oxidação;
- Desenvolver metodologia para avaliação da taxa de liberação do princípio ativo a partir de comprimidos contendo dronedarona.

REVISÃO DA LITERATURA

3 REVISÃO DA LITERATURA

3.1 Considerações gerais

Alguns tipos de disfunções cardíacas não ocorrem apenas como resultado de anormalidades do músculo cardíaco, mas também devido ao ritmo cardíaco anormal (HALL; GUYTON, 2006). A fibrilação atrial é a perturbação do ritmo cardíaco mais encontrada, sendo responsável por cerca de um terço das hospitalizações devido a distúrbios cardíacos (GO et al., 2001; FUSTER et al., 2006). Segundo Fuster e colaboradores (2006, p. e263), a fibrilação atrial pode ser definida como "uma taquiarritmia supraventricular caracterizada pela ativação atrial descoordenada com consequente deterioração da função mecânica atrial". A prevalência da fibrilação atrial aumenta com a idade. Aproximadamente 4% da população com 60 anos ou mais e 9% da população com 80 anos ou mais tem sido diagnosticadas com FA. Estima-se que, no ano de 2050, aproximadamente 5,6 milhões de adultos americanos terão FA e 88% destes pacientes terão 65 anos ou mais (GO et al., 2001). Nos últimos 20 anos, houve um aumento de 66% nas hospitalizações devido à fibrilação atrial nos Estados Unidos e União Europeia. Este aumento pode ser atribuído a combinação de fatores como a idade da população, ao aumento da prevalência de doenças cardíacas crônicas e ao diagnóstico mais frequente através do monitoramento ambulatorial. Além disso, a fibrilação atrial é um problema de saúde pública com custos elevados para o sistema de saúde, principalmente devido às hospitalizações (FUSTER et al., 2006).

A fibrilação atrial é geralmente classificada em três categorias, baseada, primariamente, na duração: paroxística (conversão espontânea dentro de sete dias, geralmente em 24h), persistente (duração maior do que sete dias) e permanente (geralmente de longa duração e não interrompida devido à falha na cardioversão elétrica ou quando não há tentativa de cessação) (FUSTER et al., 2006; YALTA et al., 2009).

As arritmias cardíacas podem variar de manifestações clínicas assintomáticas e incidentais até anormalidades potencialmente fatais. Os mecanismos subjacentes às arrítmicas cardíacas foram identificados em experimentos celulares e em animais. Em condições fisiológicas, o impulso cardíaco normal origina-se no nodo sinusal. A propagação do impulso no coração depende de dois fatores: a magnitude da corrente despolarizante (habitualmente

uma corrente de sódio) e da geometria das conexões célula a célula. Após deixar o nodo sinusal, o impulso se propaga através dos átrios, produzindo a sístole atrial. A propagação torna-se lenta quando passa pelo nodo atrioventricular (AV), onde a corrente de influxo é menor que a corrente de sódio dos átrios. Uma vez tenham deixado o nodo AV, os impulsos entram em um sistema de condução de His-Purkinje, onde as correntes de sódio são maiores que qualquer outro tecido, e os impulsos estimulam a contração ventricular coordenada (FLÓREZ, 2002; BRUNTON; LAZO; PARKER, 2006). Quando a célula cardíaca se despolariza até um determinado nível, produz-se uma resposta elétrica a qual denominamos potencial de ação cardíaco. As células cardíacas despolarizam-se e repolarizam-se para formar os potenciais de ação cardíacos aproximadamente 60 vezes por minuto. A forma e a duração de cada potencial de ação são determinadas pela atividade dos complexos proteicos que constituem os canais iônicos existentes nas membranas de cada célula. Assim, cada batimento cardíaco resulta do comportamento eletrofisiológico altamente integrado de múltiplas proteínas existentes sobre múltiplas células cardíacas. A função dos canais cardíacos pode ser perturbada por isquemia aguda, estímulos simpáticos ou pela cicatrização do miocárdio, de modo a criar anormalidades no ritmo cardíaco ou arritmias (BRUNTON; LAZO; PARKER, 2006).

As alterações do ritmo cardíaco são resultados de anomalias: a) na iniciação e propagação do impulso cardíaco (intensificação do automatismo) e b) na sequência de ativação do miocárdio (alteração na condução ou reentrada) (FLÓREZ, 2002). A intensificação do automatismo pode ocorrer em células que normalmente exibem despolarização diastólica (os nodos sinusal e AV e o sistema de His-Purkinje). Essas células são capazes de se autoexcitarem e gerar uma forma espontânea de potenciais de ação. Esse automatismo anormal pode ser produzido pela despolarização das células ventriculares (por exemplo, isquemia). Quando os impulsos se propagam para uma região onde o automatismo é anormal, ocorre arritmia. Já a reentrada pode ocorrer quando os impulsos se propagam por mais de uma via entre dois pontos do coração e quando essas vias têm propriedades eletrofisiológicas heterogêneas. Em condições normais, um impulso gerado no nodo sinusal estimula o miocárdio apenas uma vez. Em circunstâncias patológicas, um impulso pode re-excitar duas ou mais vezes o miocárdio, que é o mecanismo responsável pelas taquiarritmias clínicas (BRUNTON; LAZO; PARKER, 2006).

Uma causa frequente da fibrilação atrial é o aumento do átrio, resultante de lesões nas válvulas cardíacas, que previne o esvaziamento adequado dos átrios para dentro dos ventrículos ou uma falha ventricular devido ao excesso de sangue nos átrios. As paredes

dilatadas dos átrios geram as condições ideais para que a condução seja mais lenta e esses fatores aumentam a probabilidade de ocorrência da fibrilação atrial (NATTEL, 2002; HALL; GUYTON, 2006).

A importância clínica da fibrilação atrial está associada com a perda da contratilidade atrial, resposta ventricular acelerada e inapropriada e esvaziamento deficiente do apêndice atrial que favorecem a estase atrial. Assim, predispõe a um aumento significativo do risco de formação de trombos e por consequência de episódios tromboembólicos (FAREH, 1998).

Atualmente, o tratamento dos pacientes com fibrilação atrial envolve duas estratégias principais: controle do ritmo cardíaco e controle da frequência cardíaca. A estratégia do controle do ritmo tem por objetivo restaurar e manter o ritmo cardíaco normal, enquanto a de controle da frequência cardíaca objetiva controlar a frequência ventricular. Devido à alta taxa de recorrência da fibrilação atrial, na maioria dos pacientes, há a necessidade de um tratamento contínuo com antiarrítmicos para a manutenção do ritmo cardíaco normal (SINGH; ALIOT, 2007).

Os fármacos antiarrítmicos utilizados no tratamento da fibrilação atrial são geralmente classificados em quatro categorias baseadas em suas propriedades eletrofisiológicas: bloqueadores de canais de sódio (classe I), bloqueadores dos receptores beta-adrenérgicos (classe II), bloqueadores de canais de potássio (classe III) e bloqueadores de canais de cálcio (classe IV) (BRUNTON; LAZO; PARKER, 2006). De acordo com o esquema de Vaughan Williams, as classes I e III são geralmente utilizadas para o controle do ritmo cardíaco e as classes II e IV para o controle da frequência cardíaca (LAUGHLIN; KOWEY, 2008), devido às suas propriedades de bloqueio do nodo AV (SINGH; ALIOT, 2007).

Os agentes da classe III (amiodarona, dofetilida, ibutilida, sotalol) inibem o influxo de potássio através dos canais de potássio rápidos e ultrarrápidos, o que reduz as alterações dos impulsos cardíacos e interfere no circuito de reentrada associados com a fibrilação atrial (CHENG, 2010). A amiodarona é efetiva no tratamento de arritmias ventriculares e supraventriculares e na prevenção de morte por cardiomiopatia não isquêmica. Além disso, é o agente antiarrítmico mais frequentemente prescrito para o tratamento da fibrilação atrial na prática clínica. Entretanto, a amiodarona causa uma série de efeitos colaterais extra cardíacos como disfunção tireoidiana e toxicidade pulmonar, hepática, no sistema nervoso periférico, ocular e cutânea. Esses efeitos adversos têm sido atribuídos à presença do iodo na estrutura química da amiodarona, que torna a molécula mais lipofílica e aumenta a sua distribuição em locais do organismo onde a toxicidade é bem descrita (SUN et al., 2002; GOLDSTEIN; STAMBLER, 2005; LAUGHLIN; KOWEY, 2008).

A dronedarona é um derivado benzofurano estruturalmente relacionado com a amiodarona. Esse novo agente antiarrítmico oral foi especificamente desenvolvido para superar os efeitos adversos do seu composto relacionado, amiodarona, através da omissão da porção iodo de sua estrutura química, com a finalidade de reduzir o risco de toxicidade tireoidiana e pulmonar. Outra alteração estrutural, a adição de um grupo sulfonil-metano, reduziu a lipofilicidade da dronedarona e consequentemente, a sua meia-vida (para aproximadamente 24h), além de reduzir a acumulação tecidual (SUN et al., 1999; SUN et al., 2002; WEGENER et al., 2006; ZIMETBAUM et al., 2009). Esses fatores geraram expectativas de que a dronedarona possa funcionar como uma alternativa segura para o tratamento de pacientes com fibrilação atrial (SINGH et al., 2010).

A dronedarona é utilizada para manter o ritmo cardíaco normal após cardioversão elétrica em pacientes adultos clinicamente estáveis com história ou presença atual de fibrilação atrial não permanente, classificadas como persistente ou paroxística, de forma a prevenir a recorrência da fibrilação atrial (EMA, 2009; EMA, 2012). De acordo com o U.S. Food and Drug Administration (FDA) (2011), a dronedarona é indicada para reduzir o risco de hospitalização relacionada à fibrilação atrial em pacientes com ritmo cardíaco normal e com histórico de fibrilação atrial classificada como paroxística ou persistente.

A dronedarona é contraindicada nas seguintes condições: pacientes com fibrilação atrial permanente (pacientes nos quais o ritmo cardíaco normal não pode ser reestabelecido), pacientes com insuficiência cardíaca descompensada recente ou insuficiência cardíaca classe IV, bloqueio atrioventricular de segundo e terceiro graus (exceto quando utilizada em conjunto com um marca-passo artificial), bradicardia, uso concomitante com inibidor forte do citocromo P450 (CYP) 3A4, o uso concomitante de medicamentos ou produtos naturais que prolongam o intervalo QT e podem induzir Torsade de Pointes, toxicidade hepática ou pulmonar relacionada com o uso prévio de amiodarona, insuficiência hepática grave, gravidez, disfunção sistólica ventricular esquerda e insuficiência renal grave (FDA, 2011; EMA, 2012).

Em 2009, a aprovação deste novo agente antiarrítmico foi anunciada pelo FDA, pela European Medicines Agency (EMA) e pela Agência Nacional de Vigilância Sanitária (ANVISA). A dronedarona foi descoberta e desenvolvida pelo laboratório Sanofi-Aventis e atualmente está disponível comercialmente apenas nos Estados Unidos e na União Europeia, sob o nome comercial de Multaq®.

3.2 Estrutura química

O cloridrato de dronedarona (SR33589B) é denominado quimicamente N-{2-butil]-3-[4-(3-dibutil-aminopropóxi) benzoil] benzofurano-5-il}metanosulfonamida, cloridrato]). Sua fórmula empírica é $C_{31}H_{44}N_2O_5S$ HCl, com peso molecular relativo de 593,2. A dronedarona é um pó fino branco que é praticamente insolúvel em água e livremente solúvel em cloreto de metileno e metanol (FDA, 2011). Em sua fórmula estrutural, os grupos etila na porção nitrogênio terminal que existiam na estrutura da amiodarona foram substituídos por grupos butila, os átomos de iodo foram removidos e um grupamento metanosulfonil foi adicionado ao anel benzofurano (SUN et al., 1999, 2002).

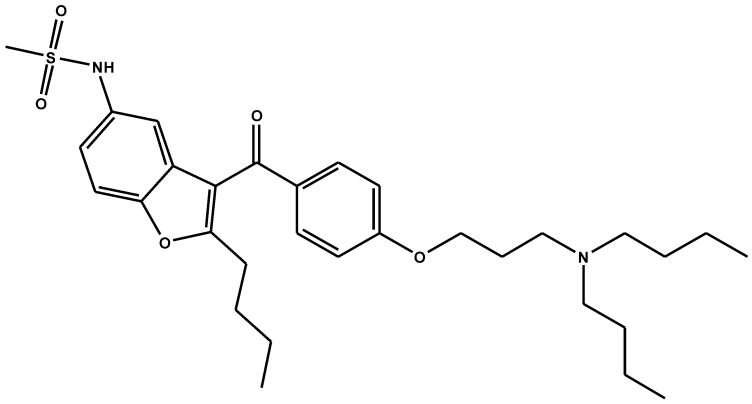

Figura 1 – Estrutura química da dronedarona.

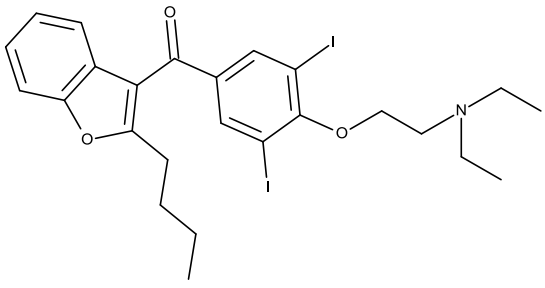

Figura 2 – Estrutura química da amiodarona.

O produto farmacêutico é fornecido na forma de comprimidos revestidos, ovais, brancos, contendo 400 mg de dronedarona (como cloridrato) para administração oral, sob o nome comercial Multaq® (EMA, 2009).

3.3 Farmacocinética

A dronedarona é bem absorvida (70 a 94%) após administração oral e a absorção aumenta de duas a quatro vezes quando administrada concomitantemente com alimentos. Entretanto, devido ao metabolismo de primeira passagem, a sua biodisponibilidade absoluta é baixa: aproximadamente 15% no estado alimentado e 4% em jejum. Após administração oral no estado alimentado, as concentrações plasmáticas de dronedarona e do seu principal metabólito ativo são alcançadas dentro de 3 a 6 horas. Com a administração repetida de 400 mg duas vezes ao dia, o estado de equilíbrio é alcançado dentro de 4 a 8 dias de tratamento, sendo que a mediana da concentração plasmática máxima de dronedarona é de 84 a 147 ng/mL e a exposição ao seu metabólito principal N-debutil é semelhante à exposição ao composto que lhe deu origem (FDA, 2011; EMA, 2012).

A ligação da dronedarona e seu principal metabólito às proteínas plasmáticas é 99,7% e 98,5%, respectivamente, e não saturável. A dronedarona é extensivamente metabolizada, principalmente pelo CYP3A4. A via metabólica predominante inclui a N-debultilação para formar o metabólito ativo principal, seguida de oxidação, desaminação oxidativa para formar o metabólito inativo ácido propanoico, seguida de oxidação e oxidação direta. O metabólito N-debutil apresenta atividade farmacodinâmica e contribui para a atividade farmacológica da dronedarona em seres humanos (FDA, 2011; EMA, 2012).

A dronedarona é excretada nas fezes principalmente na forma de metabólitos e possui meia-vida de eliminação terminal de aproximadamente 25 a 30 horas, sendo a do seu metabólito N-debutil de aproximadamente 20 a 25 horas (YALTA et al., 2009; FDA, 2011; EMA, 2012).

A dronedarona é um substrato e inibidor moderado do CYP3A4 e um fraco inibidor do CYP 2D6. Consequentemente, não deve ser administrada com potentes inibidores do CYP 3A4 como antifúngicos, antibióticos macrolídeos ou inibidores da protease. Quando coadministrada com inibidores moderados do CYP3A4 como verapamil ou diltiazem devem ser usadas doses menores dos fármacos concomitantes para evitar bradicardia e bloqueio da

condução (PATEL et al., 2009; FDA, 2011; EMA, 2012). A dronedarona, como a amiodarona, causa inibição parcial do transporte tubular de creatinina, o que resulta em uma redução da depuração de creatinina de 18 % (TSCHUPPERT et al., 2007).

3.4 Mecanismo de ação

A dronedarona previne a ocorrência de fibrilação atrial ou restaura o ritmo cardíaco normal (EMA, 2012). Em modelos animais, demonstra um efeito preventivo de taquicardia e fibrilação ventricular devido aos seus efeitos eletrofisiológicos cardíacos semelhantes à amiodarona (SUN et al., 1999; VARRÓ et al., 2001; SUN et al., 2002; GAUTIER et al., 2003). Esses efeitos se devem as suas propriedades eletrofisiológicas de um agente antiarrítmico de classe III, como a amiodarona, embora ambos os agentes demonstrem atividades eletrofisiológicas pertencentes a todas as quatro classes de Vaughan-Williams, pois são bloqueadores multicanais (GAUTIER et al., 2003; TAFRESHI; ROWLES, 2007; FDA, 2011; EMA, 2012). A dronedarona inibe as correntes de potássio (incluindo as correntes de potássio regeneradoras rápidas), prolongando assim o potencial de ação cardíaco e os períodos refratários (curto período de tempo em que a célula não pode ser reestimulada (VARRÓ et al., 2001; SUN et al., 2002). Esse agente antiarrítmico também inibe as correntes de sódio (Classe Ib) (LALEVÉE et al., 2003) e as correntes de cálcio (Classe IV) (GAUTIER et al., 2003; BOGDAN et al., 2011; EMA, 2012). Além disso, ele é um agente antagonista dos adrenorreceptores alfa e beta (Classe II) (HODEIGE et al., 1995).

3.5 Estudos clínicos

O ATHENA (A Placebo-Controlled, Double-blind, Parallel Arm Trial to Assess the Efficacy of Dronedarone 400 mg bid for the Prevention of Cardiovascular Hospitalization or Death from Any Cause in Patients with Atrial Fibrillation/ Atrial Flutter) foi desenvolvido para determinar se a dronedarona reduziria a taxa de hospitalização devido a eventos cardiovasculares ou morte em pacientes com fibrilação atrial. Os pacientes com fibrilação atrial paroxística ou permanente foram selecionados de acordo com os seguintes requisitos:

hipertensão arterial, *diabetes mellitus*, acidente vascular cerebral prévio, ataque isquêmico transitório ou embolia sistêmica, diâmetro do átrio esquerdo maior ou igual a 50 mm. Os pacientes podiam estar no ritmo cardíaco normal ou em processo de fibrilação atrial. Um total de 4628 pacientes foram aleatorizados, dos quais 2301 receberam dronedarona e 2327 receberam placebo, tratados durante uma média de 22 meses (no máximo 30 meses). A média de idade dos pacientes foi de 71,6 anos e 46,9% eram do sexo feminino. A maioria dos pacientes tinha hipertensão (86%) e doença cardíaca estrutural (60%). Vinte e cinco por cento (25%) dos pacientes apresentavam fibrilação atrial no início do estudo. A dronedarona reduziu em 24,2% a incidência de hospitalização por eventos cardiovasculares ou morte por qualquer causa, em comparação com o placebo. Além disso, reduziu o risco de hospitalização cardiovascular em 25,5%. O número de mortes por qualquer causa foi comparável entre o grupo da dronedarona (5% dos pacientes) e o grupo placebo (6%). Houve 63 mortes (2,7% dos pacientes) por eventos cardiovasculares no grupo da dronedarona e 90 mortes (3,9% dos pacientes) do grupo placebo, 26 mortes devido à arritmia cardíaca (1,1% dos pacientes) no grupo da dronedarona e 48 (em 2,1%) no grupo placebo. O estudo concluiu que a terapia com dronedarona conduziu a uma redução significativa na incidência de hospitalização cardiovascular ou morte em comparação ao placebo em pacientes com FA (HOHNLOSER et al., 2009).

Os estudos clínicos EURIDIS (European Trial in Atrial Fibrillation or Flutter Patients Receiving Dronedarone for the Maintenance of Sinus Rhythmic) e ADONIS (American-Australian- African Trial with Dronedarone in Atrial Fibrillation or Flutter Patients for the Maintenance of Sinus Rhythmic) foram idênticos, controlados por placebo, multicêntricos, duplo-cego e com grupos paralelos. A eficácia da dronedarona foi avaliada em um total de 1237 pacientes randomizados, com 828 pacientes recebendo 400 mg de dronedarona duas vezes ao dia e 409 recebendo placebo. Os pacientes selecionados tiveram pelo menos um episódio de fibrilação atrial nos últimos 3 meses. O ritmo cardíaco foi monitorado por ECG por até 12 meses. O primeiro *endpoint* foi o tempo decorrido até a primeira recorrência de fibrilação atrial. Nos resultados dos dois estudos combinados, a mediana do tempo da recorrência de fibrilação atrial foi de 116 dias para o grupo da dronedarona e 53 para o grupo placebo. Ao final dos 12 meses, a taxa de recorrência foi de 64,1% para o grupo da dronedarona e 75,2% para o grupo placebo. Os resultados dos experimentos demonstraram que a taxa da primeira recorrência de fibrilação atrial e da primeira recorrência sintomática em 1 ano foi significativamente reduzida com o uso da dronedarona em comparação com o placebo (SINGH et al., 2007).

O estudo clínico ANDROMEDA (Antiarrhythmic Trial with Dronedarone in Moderate to Severe CHF Evaluating Morbidity Decrease) foi realizado com pacientes hospitalizados com novo caso ou com agravamento de insuficiência cardíaca os quais tiveram pelo menos um caso de dispneia em repouso ou após mínimo esforço (classe funcional III ou IV da classificação da New York Heart Association) ou dispneia paroxística noturna durante o mês anterior a sua admissão no estudo. Neste estudo foram incluídos 627 pacientes (310 no grupo da dronedarona e 317 no grupo placebo), o qual foi interrompido prematuramente após 7 meses por questões de segurança, devido ao número de mortes ocorridas no grupo da dronedarona (25 pacientes) em comparação com o grupo placebo (12 pacientes). Além disso, durante o estudo o número total de pacientes que tiveram a primeira hospitalização devido a eventos cardiovasculares agudos foi maior no grupo da dronedarona (71 pacientes) do que no grupo placebo (50 pacientes). O estudo concluiu que o tratamento com dronedarona em pacientes com insuficiência cardíaca severa e disfunção sistólica ventricular esquerda resultou em um aumento prematuro da mortalidade e, por isso a dronedarona não deve ser administrada em pacientes com insuficiência cardíaca e disfunção sistólica ventricular esquerda (KOBER et al., 2008).

O estudo clínico DIONYSOS (Randomized, Double-blind TrIal to Evaluate the Efficacy and Safety of DrOnedarone [400 mg bid] Versus Amiodarone [600 mg qd for 28 daYs, then 200 mg qd Thereafter] for at Least 6 mOnths for the Maintenance of Sinus Rhythm in Patients with AF) é um estudo de curto prazo comparando a dronedarona (400 mg duas vezes ao dia) com a amiodarona (600 mg diários durante 28 dias, depois 200 mg diários nos dias subsequentes) durante 6 meses. O estudo foi conduzido a fim de fornecer uma comparação entre a relação risco benefício de dronedarona e amiodarona. O objetivo primário foi definido como a recorrência de FA ou a descontinuação do estudo do fármaco devido a intolerância ou falta de eficiência em 12 meses. O estudo foi iniciado em pacientes ainda em FA com a finalidade de permitir a análise de intenção de tratar. Um total de 504 pacientes foram aleatorizados, 249 pacientes no grupo da dronedarona e 255 pacientes no grupo da amiodarona. A maioria dos pacientes tinha hipertensão (67%) e histórico de FA persistente (63%). A incidência do objetivo primário de eficácia definido como primeira recorrência de FA ou a descontinuação do estudo do fármaco devido a intolerância ou falta de eficácia aos 12 meses foi de 75,1% no grupo da dronedarona e 58,8% no grupo da amiodarona, devido principalmente a recorrência de FA que foi mais frequente no grupo da dronedarona (63,5%) do que no grupo da amiodarona (42%). Entretanto, as descontinuações prematuras do fármaco devido a intolerância foram mais frequentes no grupo da amiodarona (13,3%) em comparação

a dronedarona (10,4%). A incidência do objetivo primário de segurança (primeira ocorrência de eventos específicos da tireoide, hepáticos, pulmonares, neurológicos, cutâneos, oculares ou gastrintestinais ou a descontinuação prematura do estudo seguida de efeitos adversos) foi de 39,3% e 44,5% para o grupo da dronedarona e amiodarona, respectivamente, ao fim dos 12 meses de tratamento. A redução desses efeitos no grupo da dronedarona em relação ao grupo da amiodarona deve-se a uma diminuição dos eventos na tireoide, neurológicos, cutâneos e oculares. 9,2% dos pacientes do grupo da dronedarona e 3,1% dos pacientes do grupo da amiodarona tiveram pelo menos um episódio de diarreia. Se os efeitos adversos gastrintestinais não fossem considerados, haveria uma redução significativa (39%) dos efeitos adversos em favor da dronedarona em relação à amiodarona. Nesse estudo, a dronedarona demonstrou ser menos efetiva em relação à amiodarona na redução da recorrência de FA, entretanto obteve um perfil melhor de segurança, especificamente com relação à tireoide e eventos neurológicos (LE HEUZEY et al., 2010).

O PALLAS (Permanent Atrial Fibrillation Outcome Study Using Dronedarone on Top of Standard Therapy) é um estudo clínico randomizado, duplo-cego, controlado por placebo e concebido para testar se a dronedarona poderia reduzir as taxas dos principais eventos vasculares ou hospitalização cardiovascular não planejada em pacientes com fibrilação atrial permanente que estão com alto risco para eventos vasculares. Foram selecionados pacientes com fibrilação atrial permanente com idade igual ou superior a 65 anos e pelo menos um dos fatores de risco: doença arterial coronariana, acidente vascular cerebral prévio, ataque isquêmico transitório, insuficiência cardíaca sintomática, fração de ejeção do ventrículo esquerdo de 40% ou menos, doença arterial periférica, ou a combinação de fatores da idade de 75 anos ou mais, hipertensão e diabete. Os pacientes receberam uma dose de 400 mg de dronedarona duas vezes ao dia ou placebo. Um total de 3236 pacientes tinham sido submetidos a randomização com acompanhamento médico de 3,5 meses (dronedarona = 1619, placebo = 1617). A média de idade dos pacientes foi de 75 anos e 69% tinham um histórico de mais de dois anos de fibrilação atrial permanente. Dois terços dos pacientes tinham histórico de insuficiência cardíaca e 88% estavam recebendo mediação para o controle da frequência cardíaca. O estudo foi terminado prematuramente devido ao aumento significativo de insuficiência cardíaca, da mortalidade e da hospitalização cardiovascular não planejada. Houve 25 mortes no grupo da dronedarona e 13 no grupo placebo; 21 mortes foram associadas a causas cardiovasculares no grupo da dronedarona e 10 no grupo placebo. Acidente vascular cerebral ocorreu em 23 pacientes no grupo da dronedarona e 10 no grupo placebo. Em 113 pacientes recebendo dronedarona ocasionou hospitalização cardiovascular

não planejada, contra os 59 pacientes recebendo o placebo; hospitalizações devido a insuficiência cardíaca ocorreram em 43 pacientes do grupo da dronedarona e em 24 pacientes do grupo placebo; episódios de insuficiência cardíaca ocorreram em 115 pacientes recebendo dronedarona e em 55 pacientes recebendo placebo. A dronedarona aumentou as taxas de insuficiência cardíaca, acidente vascular cerebral e morte devido a causas cardiovasculares em pacientes com fibrilação atrial permanente, os quais estão em maior risco de eventos vasculares. Os dados desse estudo demonstram que a dronedarona não deve ser utilizada nesses pacientes (CONNOLLY et al., 2011).

3.6 Métodos de determinação de dronedarona

Não há monografias disponíveis em compêndios oficiais ou metodologias validadas na literatura para avaliação de dronedarona em forma farmacêutica ou matéria-prima.

Na literatura científica, há somente um método descrito para a determinação de dronedarona e seu metabólito principal em materiais biológicos. Bolderman e colaboradores (2009) desenvolveram um método para determinação dos antiarrítmicos dronedarona e amiodarona e seus principais metabólitos em plasma e em tecido muscular (miocárdio) por cromatografia líquida de alta eficiência acoplada à detecção UV. A separação foi realizada em coluna polimérica Pathfinder PS C18 (50 mm x 4,6 mm, 2,5 μm) (Shimadzu, Kyoto, Japão). A corrida cromatográfica de 10 minutos foi conduzida utilizando-se fase móvel composta por acetonitrila, isopropanol, água e amônia (80/10/10/0,025, v/v/v/v) com fluxo de 1,0 mL/ min. O método proposto foi validado e permitiu a detecção seletiva de amiodarona e dronedarona e seus respectivos metabólitos em plasma e amostras de tecido.

3.7 Validação de métodos analíticos

O desenvolvimento de métodos analíticos para a determinação de fármacos tem recebido atenção considerável devido a sua importância no controle de qualidade.

A análise de produtos farmacêuticos é realizada, geralmente, por cromatografia líquida, que é o método de referência em muitas indústrias farmacêuticas (ALTRIA, 1999). A

capacidade de alta resolução, sensibilidade e especificidade da cromatografia líquida fez desta técnica a mais empregada em estudos de estabilidade (BAKSHI; SINGH, 2002).

No entanto, para a análise quantitativa de fármacos em forma farmacêutica, o método por cromatografia líquida possui a desvantagem do alto custo, além de necessitar mais tempo para o seu desenvolvimento em comparação aos métodos espectrofotométricos, sendo estes econômicos, rápidos e requerem instrumentação simples.

A eletroforese capilar (EC) tem sido utilizada nos últimos anos como uma alternativa a cromatografia líquida devido a inúmeras vantagens como alta eficiência de separação, rapidez de análise, simplicidade do instrumento, redução do tempo para desenvolvimento de método analítico e redução nos custos de operação e no consumo de solventes (ALTRIA, 1999; NISHI, 1999). A eletroforese capilar é um método analítico com uma ampla variedade de aplicações, tais como análises físico-químicas de fármacos, excipientes e impurezas relacionadas em produtos farmacêuticos; separação quiral; análises biológicas e forenses (ALTRIA et al., 2006; HOLZGRABE et al., 2006). A cromatografia eletrocinética micelar (MEKC) é uma tipo de separação cromatográfica baseada na solubilização micelar e migração eletrocinética, realizada através da dissolução de um tensoativo iônico na solução eletrolítica em uma concentração maior do que a concentração micelar crítica. O mecanismo da separação é baseado na partição do analito entre a fase micelar e a solução eletrolítica; assim, a fase micelar é chamada de pseudo-estacionária, pois corresponde a fase estacionária da cromatografia convencional. A MEKC é um modo de separação versátil, pois é otimizada sem a necessidade de modificações instrumentais no equipamento de EC e é utilizada para a separação de moléculas neutras como compostos insolúveis em água, os quais não podem ser separados pela eletroforese capilar de zona (TERABE et al., 1984; OTSUKA et al., 1985; KIM et al., 2001; TERABE, 2004; TERABE, 2010).

A validação de um procedimento analítico tem, por objetivo, demonstrar que o mesmo é adequado para a análise pretendida (ICH, 2005). Segundo a USP 34 (2011) a validação é um processo no qual se estabelece, através de estudos de laboratório, que as características de desempenho do método satisfazem as exigências para a aplicação analítica pretendida. De acordo com a Resolução RE n° 899 da ANVISA (BRASIL, 2003), que dispõe de um guia para validação de métodos analíticos e bioanalíticos, a validação deve garantir, através de estudos experimentais, que o método atenda às exigências analíticas, assegurando a confiabilidade dos resultados. Essa resolução ainda ressalta que os equipamentos devem estar calibrados e os analistas devem ser qualificados e treinados.

O ICH (2005) divide os métodos analíticos em quatro categorias, incluindo os testes: de identificação, quantitativos de impurezas, para controle do limite de impurezas e os ensaios para a determinação quantitativa, a fim de avaliar a potência (teor) do fármaco ou para a verificação do perfil de dissolução de amostras específicas.

No presente trabalho, a validação foi conduzida conforme o ICH (2005), a Resolução RE n° 899 da ANVISA (BRASIL, 2003) e USP 34 (2011). Os parâmetros de validação utilizados foram os seguintes: especificidade, linearidade, precisão, exatidão, robustez, limite de quantificação e limite de detecção. A especificidade é a capacidade do método analítico em medir exatamente um analito na presença de todos os potenciais componentes da amostra, tais como impurezas e produtos de degradação. A linearidade é a habilidade do método em obter resultados que são diretamente proporcionais à concentração do analito na amostra dentro de um dado intervalo. A precisão de um método analítico expressa o grau de proximidade dos resultados obtidos entre uma série de medidas obtidas de uma amostragem múltipla de uma mesma amostra homogênea. Geralmente é expressa como desvio padrão relativo (DPR) e é considerada em três níveis: repetibilidade, precisão intermediária e reprodutibilidade. A repetibilidade é expressa pelos resultados obtidos dentro de um curto período de tempo e sob as mesmas condições (precisão intra-dia), verificada por um mínimo de nove determinações, abrangendo o intervalo linear do método (três concentrações com três réplicas cada) ou no mínimo seis determinações a 100% da concentração do teste. A precisão intermediária é determinada observando-se a concordância dos resultados obtidos a partir de variações dentro do laboratório como dias diferentes, analistas, equipamentos, etc. A reprodutibilidade é avaliada pela análise de amostras homogêneas por laboratórios diferentes como em estudos colaborativos. A exatidão é a proximidade dos valores obtidos pelo método analítico em relação ao valor verdadeiro. A exatidão pode ser determinada através da recuperação de quantidades conhecidas do analito (padrão de referência), as quais foram adicionadas ao medicamento. A robustez de um método analítico é a sua capacidade de resistir a pequenas e deliberadas variações nos parâmetros do método, que deve ser considerada durante o desenvolvimento do método, pois indica a sua confiança durante o uso normal. O limite de detecção de um método analítico é a menor quantidade do analito em uma amostra que pode ser detectada, mas não necessariamente quantificada. O limite de quantificação é definido como a menor concentração do analito em uma amostra que pode ser determinada quantitativamente com precisão e exatidão aceitáveis. O teste de adequabilidade do sistema é utilizado para verificar se a resolução e a repetibilidade do sistema estão adequadas para a realização de uma análise. O teste tem, por base, o conceito que

equipamentos, experimentos e amostras constituem um sistema integrado que pode ser avaliado como um todo (BRASIL, 2003; SHABIR, 2003; ICH, 2005; USP, 2011).

Para validar um método indicativo de estabilidade, o teste de degradação forçada deve ser realizado sob uma ampla variedade de condições de estresse, a fim de fornecer a análise dos produtos de degradação individuais. Além disso, pode também avaliar as características de estabilidade inerentes ao fármaco e as vias de degradação, proporcionando assim informação relevante da estabilidade do fármaco (BAKSHI; SINGH, 2002; ICH, 2003).

3.8 Teste de dissolução

O estudo de dissolução é o método mais importante para avaliar, em condições *in vitro*, a liberação de um fármaco a partir de uma forma farmacêutica sólida. Representa, assim, uma ferramenta indispensável para prever os fatores que podem influenciar a biodisponibilidade do fármaco. O teste de dissolução é de uma natureza física destrutiva, no qual o fármaco passa para a forma solúvel a partir da forma farmacêutica intacta (ABDOU, 1989; BANAKAR, 1992). Os testes de dissolução foram desenvolvidos para garantir a qualidade lote-a-lote, guiar o desenvolvimento de novas formulações, assegurar a qualidade e performance do fármaco após determinadas alterações e indicar problemas potenciais de biodisponibilidade (FDA, 1997). Durante o teste de dissolução, a velocidade de liberação do fármaco pode ser avaliada, relacionando-se a quantidade cumulativa do fármaco liberada na solução com o tempo *t*. O objetivo do teste de dissolução é encontrar uma característica *in vitro* de uma formulação em potencial que reflita a sua performance *in vivo* (AULTON, 2005). O estabelecimento de correlações *in vitro*/*in vitro* é vantajoso do ponto de vista ético, econômico e técnico, pois pode substituir ou reduzir os estudos de bioequivalência em seres humanos (COSTA; LOBO, 2001). Por esse motivo, o planejamento de um teste de dissolução necessita reproduzir as condições fisiológicas do trato gastrintestinal após a administração da forma farmacêutica (DRESSMAN, 1998). A solubilidade de um fármaco é um parâmetro relevante na escolha do meio de dissolução, a qual pode ser determinada em diferentes meios e expressa como a quantidade de fármaco dissolvido (mg/mL) (ABDOU, 1989; BROWN, 2004).

Os capítulos seguintes descrevem os métodos analíticos desenvolvidos neste trabalho, nos quais estão descritos os materiais e métodos que foram utilizados e os resultados obtidos.

ARTIGO 1: DESENVOLVIMENTO E VALIDAÇÃO DE MÉTODOS POR CL-FR E ESPECTROFOTOMETRIA NO UV PARA A ANÁLISE DE DRONEDARONA EM COMPRIMIDOS

4 ARTIGO 1 – DESENVOLVIMENTO E VALIDAÇÃO DE MÉTODOS POR CL-FR E ESPECTROFOTOMETRIA NO UV PARA A ANÁLISE DE DRONEDARONA EM COMPRIMIDOS

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Development and Validation of Stability-Indicating RP-LC and UV-Spectrophotometric Methods for the Analysis of Dronedarone in Tablets

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Running title: Dronedarone reversed phase liquid chromatography spectrophotometry

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Abstract:

Two analytical methods have been developed for the determination of dronedarone in pharmaceutical formulation. The first method is the stability-indicating reversed-phase liquid chromatography (RP-LC). This method was carried out on a C_{18} column (250 mm x 4.6 mm i.d.), maintained at room temperature, using a buffer solution (0.3% glacial acetic acid; pH 4.9): acetonitrile (35:65, v/v) as the mobile phase, running at a flow rate of 1.0 mL min^{-1}. Photodiode array detector was used to obtain peak purity indices (> 0.9998) and the detection wavelength was 289 nm. Chromatographic separation was obtained within 7.0 min. Dronedarone was exposed to stress conditions of acid and basic hydrolysis, oxidation, thermal and photodegradation and no interference from the degradation products was detected. The dronedarone degradation kinetics in alkaline conditions was also studied and followed apparent first-order reaction. The second method is a simple and rapid ultraviolet (UV) spectrophotometric method, which was developed as a low-cost alternative to assay dronedarone in film-coated tablets. Both methods were validated with respect to specificity, linearity, precision, accuracy, limit of detection, limit of quantitation and robustness according to ICH guidelines. The methods were linear in the range from 5.0 to 100.0 µg mL^{-1} (r = 0.9999) for the RP-LC method and in the range from 5.0 to 25.0 µg mL^{-1} for the UV method. Moreover, validation parameters demonstrated acceptable results. The methods proposed were successfully applied for the quantitative analysis of dronedarone in pharmaceutical dosage form assuring therapeutic efficacy.

Keywords: dronedarone, reversed phase liquid chromatography, UV spectrophotometry, validation.

1.Introduction

Dronedarone HCl (DRO; SR33589B; Fig. 1) is chemically described as N-{2-butyl-3-[4-(3-dibutylaminopropoxy) benzoyl] benzofuran-5-yl} methanesulfonamide, hydrochloride. Its empirical formula is $C_{31}H_{44}N_2O_5$ S HCl with molecular weight of 593.2 [1]. Dronedarone is a noniodinated benzofuran derivative structurally and pharmacologically related to amiodarone, developed as an antiarrhythmic agent to overcome the side effects of its parent compound [2-4]. The molecular changes made to amiodarone to produce dronedarone included the removal of iodine moiety and the addition of a methane-sulfonyl group, which conferred less lipophilic character (associated with reduced accumulation in tissue) and less thyroid toxicity [3,5-6].

In July 2009, the U.S. Food and Drug Administration (FDA) approved dronedarone to reduce the risk of cardiovascular hospitalization in patients with atrial fibrillation [1]. Dronedarone inhibits sodium and L-type calcium inward currents and potassium outward currents, and thus prolonging cardiac action potential and refractory periods [4-5]. Furthermore, dronedarone possess a non-competitive antiadrenergic action that may be partly responsible for the antiarrhythmic action [2].

The literature survey revealed only one paper dealing with determination of dronedarone in biological matrices. High-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV) for the determination of dronedarone and its main metabolite debutyldronedarone in both plasma and myocardial tissue has been reported using acetonitrile, isopropanol, water and ammonia (80/10/10/0.025, v/v/v/v) as mobile phase at flow rate of 1.0 mL min^{-1} [7].

However, no stability-indicating analytical methods have been reported in literature for the determination of dronedarone in bulk form or pharmaceutical dosage form and there is no information regarding the stability behavior of the drug under stress conditions. Moreover, none of the most recognized pharmacopoeias include official methods for its assay in the pharmaceutical dosage form.

In order to validate the stability-indicating capability of an analytical method, stress testing should be carried out under a variety of exposure conditions, to provide the analysis of individual degradation products. Furthermore, the inherent stability characteristics of the drug and degradation pathways could also be evaluated, thus providing relevant information on drug stability [8, 9].

The high-resolution capacity, sensitivity and specificity of reversed phase liquid chromatography (RP-LC) made this technique the most widely used in stability studies [9]. However, for quantitative analysis of drugs in the pharmaceutical dosage form, RP-LC method is relatively more expensive and time-consuming than spectrophotometric methods, which have the advantage of being economic, fast and require simple instrumentation.

Therefore, the aim of the present work was to develop a simple, reliable, accurate and stability-indicating RP-LC and UV spectrophotometric methods, which have been successfully applied for the determination of dronedarone in film-coated tablets. The methods were validated according to the ICH guidelines [10].

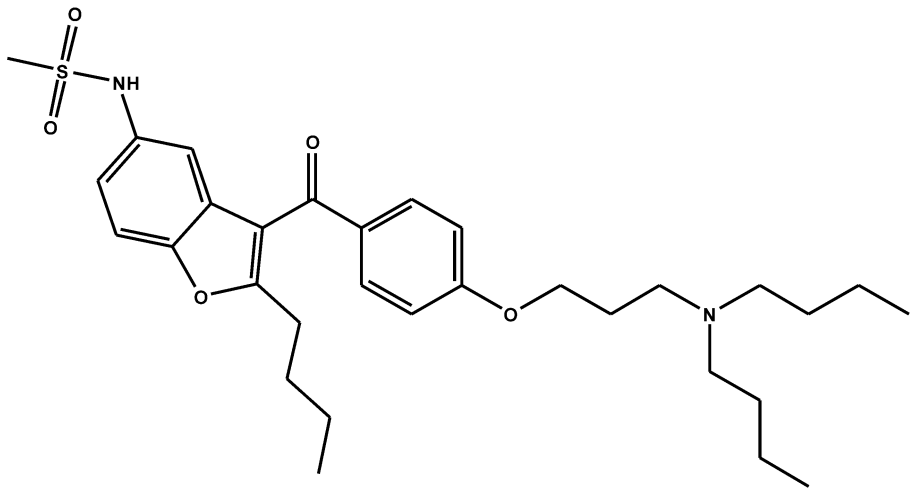

Figure 1. Chemical structure of dronedarone.

2. Experimental

2.1. Chemicals

Dronedarone hydrochloride reference standard (assigned purity 99.7%) was purchased from Sequoia Research Products (Pangbourne, Berkshire, UK). Film-coated tablets containing 400 mg of dronedarone hydrochloride (Multaq®, Sanofi-Aventis, Paris, France) were obtained from commercial sources (batch 0A048). HPLC-grade methanol and acetonitrile, glacial acetic acid and triethylamine (TEA) were obtained from Tedia Company Inc (Fairfield, OH, USA). For all analyses, ultrapure water was purified with WaterPro™PS, Labconco system

(Kansas City, MO, USA). All other chemicals were of pharmaceutical grade. Dronedarone film-coated tablet was labeled to contain the following excipients:

Core of tablets – hypromellose, starch, crospovidone, poloxamer 407, lactose monohydrate, colloidal silicon dioxide, magnesium stearate.

Coating / polishing of tablets – hypromellose, polyethylene glycol 6000, titanium dioxide, carnauba wax.

2.2. Instrumentation and analytical conditions

2.2.1. RP-LC method

The RP-LC method was performed on a Shimadzu LC system (Kyoto, Japan) equipped with CBM-20A system controller, LC-20AT pump, DGU-20A_5 degasser, SPD-M20A photodiode array (PDA) detector and Rheodyne® (Rohnert Park, CA, USA) injector model 7725i. Peak areas were automatically integrated using Shimadzu LC Solution software (version 1.24SP1). Analytical separations were performed on a new Waters (Milford, MA, USA) XBridge™ C_{18} column (250 mm × 4.6 mm i.d., 5 µm particle size).

2.2.2. UV spectrophotometry

The UV spectrophotometric method for quantitative assay of dronedarone in tablets was performed on Pró-Análise (Porto Alegre, RS, Brazil) spectrophotometer equipped with 1 cm quartz cells.

2.3. Preparation of reference solutions

RP-LC method: the stock standard solution (500 µg ml^{-1}) was prepared by dissolving 10.0 mg dronedarone reference standard in 20 mL of methanol. The stock standard solution was daily diluted in methanol up to adequate concentration and stored protected from the light at -20°C.

UV method: an accurately weighed 10 mg of dronedarone reference standard was transferred into a 20 mL volumetric flask and dissolved in methanol to obtain final concentration of 500 µg ml^{-1}. The stock solution was also diluted in methanol and stored at -20°C.

2.4. Preparation of sample solutions

RP-LC method: twenty tablets containing 400 mg of dronedarone were accurately weighed, combined and thoroughly crushed to a fine powder. An amount of tablet powder equivalent to 20 mg of dronedarone was transferred into 50 mL volumetric flasks. After diluting in 30 mL of methanol, samples were sonicated for 15 minutes. Then, the volume of flasks was filled up with methanol and the solution (containing 400 µg ml^{-1}) was filtered through quantitative filter paper (Schleicher & Schuell). An aliquot of 1 mL at concentration of 400 µg ml^{-1} was diluted in methanol to obtain final concentration of 20 µg ml^{-1} and then filtered through 0.45 µm membrane filter (Sartorius, Germany) before RP-LC analysis. The sample solutions were daily prepared.

UV method: to prepare the sample solution, an amount of tablet powder equivalent to 15 mg of dronedarone was accurately weighed and transferred into a 50 mL volumetric flask. Then, it was diluted with 30 mL of methanol and sonicated for 15 minutes. After, the volume of samples was filled up with methanol to obtain concentration of 300 µg ml^{-1} and filtered through quantitative filter paper (Schleicher & Schuell). An aliquot of 1mL was transferred into a 20 mL volumetric flask, diluted with methanol to obtain final concentration of 15 µg ml^{-1}, which was analyzed by UV-spectrophotometry. Working sample solutions were also daily prepared.

2.5. Chromatographic conditions

The RP-LC system was operated at room temperature (25 ± 1 °C). The mobile phase consisted of a buffer solution pH 4.9 (0.3% glacial acetic acid adjusted with TEA): acetonitrile (35:65, v/v), and flow rate of 1.0 mL min^{-1}. It was filtered through 0.45 µm membrane filter (Sartorius, Goettingen, Germany) and degassed before use. The injection volume was 20 µL. PDA detector was set at 289 nm.

2.6. Spectrophotometric conditions

To obtain the absorbance measurements and spectrum, methanol was used as diluent and the selected wavelength was 289 nm.

2.7. Forced degradation studies

Forced degradation studies were performed to provide the stability-indication property and specificity of the RP-LC method in accordance with the ICH regulatory guide [10]. Stress studies were performed by submitting a tablet solution (100 µg mL^{-1}) under different stress conditions such as acid, alkali, oxidation, heat and UV light, by using the proportion 1:1 (v/v; tablet solution: degradant). After degradation, aliquots of this solution were diluted with methanol to obtain final concentration of 10 µg mL^{-1}. Stressed samples were chromatographed along with non-stressed sample and the peak purity test of the degraded samples was carried out by PDA detector.

2.7.1. Hydrolytic conditions

Acid hydrolysis was performed by heating the tablet solution in 3.0 M hydrochloric acid (HCl) at 80°C for 7h. The study in alkaline conditions was carried out with 1.0 M sodium hydroxide (NaOH) at 80°C for 30 min. Then, the solutions were cooled and neutralized by adjusting the pH to 7.0 with base or acid as needed.

2.7.2. Oxidative conditions

Oxidative studies were carried out by storing the tablet solution in 30% hydrogen peroxide (H_2O_2), protected from light at room temperature for 48 h.

2.7.3. Photolytic degradation

Photodegradation was induced by placing the tablet solution in sealed quartz vessels and exposing in photostability chambers to near UV-A (352 nm) and UV-C (254 nm) light for 24 h and 1h30, respectively. Dark control samples were prepared for comparison purposes, as recommended by the ICH guideline [11]. At the end of the exposure period, samples were withdrawn and diluted with methanol to final concentrations of 10 µg mL^{-1}.

2.7.4. Thermal studies

For the study under neutral thermo degradation, the sample solution was diluted in water and heated at 80°C for 6 h.

2.8. Kinetics study

The dronedarone degradation under alkaline condition was faster, thus it was determined by diluting a DRO sample solution of (100 µg mL^{-1}) with 1.0 M NaOH and placing it in a thermostatic water bath at 60°C. After pre-established time intervals, samples were withdrawn, neutralized with 1.0 M HCl and diluted with methanol to final concentration of 20 µg mL^{-1}.

The kinetics of dronedarone degradation under basic conditions was monitored by RP-LC method and was evaluated by plotting the remaining drug (µg ml^{-1}) versus time in hours (zero-order reaction), the logarithmic of the remaining concentrations of dronedarone versus time (apparent first-order reaction) and remaining concentration $^{-1}$ versus time (second-order) to verify which presented a better fit.

2.9. Validation of the analytical methods

Validation was carried out assessing the following parameters: specificity, linearity, precision, accuracy, limits of detection and quantitation and robustness according to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [10]. The system suitability test was also carried out to evaluate the reproducibility of the chromatographic system, using five replicate injections of a reference solution. The parameters measured were injection repeatability, retention time, theoretical plates, peak symmetry and capacity factor.

2.9.1. Specificity

The specificity of the RP-LC method was evaluated by comparing the reference solution, sample solution and placebo chromatograms. The placebo solution consisted of an in-house mixture of all tablet excipients without the active ingredient prepared using the same preparation procedure of the sample solution. The specificity of the RP-LC method was also

measured by determining peak purities of stressed samples using PDA detector. The ability of the method proposed to separate dronedarone from its degradation products was evaluated through the resolution factor.

A placebo solution was analyzed by the spectrophotometric method in order to determine its specificity. For both methods, the interference from the formulation matrix was evaluated.

2.9.2. Linearity

Linearity was determined by constructing three independent analytical curves. For the RP-LC method, the analytical curves were obtained with five concentration levels of stock standard solution, ranging from 5.0 to 100.0 μg ml^{-1} (5, 10, 20, 50 and 100 μg mL^{-1}), prepared in methanol. Triplicate injections were made for each sample. The analytical curves were drawn by plotting the peak areas of chromatograms against the respective dronedarone concentrations.

To establish the linearity of the spectrophotometric method, the stock standard solution was diluted in methanol to yield concentration ranging from 5.0 to 25.0 μg ml^{-1} (5, 10, 15, 20 and 25 μg ml^{-1}).

Linearity was evaluated by linear regression analysis with the method of least squares which was used to calculate the correlation coefficient, y-intercept and slope of the regression line.

2.9.3. Precision

The precision of procedures was determined by repeatability (intra-day) and intermediate precision (inter-day), expressed as relative standard deviation (RSD). The repeatability of the RP-LC method was evaluated by injecting six independent sample preparations at the same dronedarone concentration (20 μg ml^{-1}), during the same day, under the same experimental conditions. The intermediate precision of the RP-LC method was determined by comparing the assays during three different days and also by different analysts (between-analysts) in one laboratory.

The repeatability of the UV spectrophotometric method was evaluated by carrying out six independent assays of dronedarone sample preparations at 15 μg ml^{-1} against reference

solution. The intermediate precision of the spectrophotometric method was performed by repeating the assays on three different days and using different analysts.

2.9.4. Accuracy

For the RP-LC method, the accuracy was evaluated by the recovery of known amounts of reference solution (5.0, 15.0 and 25.0 µg ml^{-1}), added to a sample solution (5.0 µg ml^{-1}) to obtain sample solutions with final concentrations of 10.0, 20.0 and 30.0 µg ml^{-1}, equivalent to 50, 100 and 150% of the nominal analytical concentration, respectively. The samples of each concentration were prepared in triplicate.

For the UV spectrophotometric method, the accuracy was demonstrated by spiking samples of dronedarone tablets (previously analyzed) with three different concentrations of the reference solution (5.0, 10.0 and 15.0 µg ml^{-1}), to give solutions at concentrations of 10.0, 15.0 and 20.0 µg ml^{-1}. The accuracy was evaluated in triplicate using three concentration levels: 66, 100 and 133% of the analytical method concentration.

For assay methods, accuracy criteria is that the mean recovery will be 100 ± 2% at each concentration [12]. The accuracy was calculated as the percentage of drug recovered from the formulation and also expressed as relative standard deviation (RSD) between measurements.

2.9.5. Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of response (σ) and slope (S) using three independent analytical curves, as defined by ICH guideline (2005): $LOD = 3.3\sigma/S$ and $LOQ = 10\ \sigma/S$. LOD and LOQ were also experimentally estimated for the LC method by determining the signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively.

2.9.6. Robustness

In order to determine the robustness of both methods, experimental conditions were deliberately changed. The robustness of the RP-LC method was performed by analyzing the same sample under deliberate changes in chromatographic conditions. The flow rate of the mobile phase was changed by 0.2 units from 0.8 to 1.2 mL/ min and buffer pH value was

changed by ± 0.5 units. The effect of the percent acetonitrile strength was also studied by varying acetonitrile in the mobile phase by ± 5%.

The stability of reference and sample solutions during RP- LC analysis was studied and both solutions were left in tightly capped flasks at room temperature for 12 and 24h. After these periods, the same solutions were analyzed and compared with a freshly prepared reference solution.

The robustness of the spectrophotometric method was determined by changing the wavelength (287-291 nm) and using different solvent brands.

2.9.7. System suitability tests

System suitability tests are an integral part of the chromatographic method, which are used to verify that the chromatographic system is adequate to the analysis to be performed [13]. The test was performed by injecting six replicates of the reference solution containing 20 µg ml^{-1} of dronedarone, following USP 34 [13]. The peak parameters evaluated were capacity factor, number of theoretical plates and tailing factor.

2.10. Analysis of dronedarone in pharmaceutical formulations

The commercial pharmaceutical preparation was analyzed by both methods in triplicate. For dronedarone quantification in the tablet formulation, samples were prepared according to procedure mentioned in Section 2.4 and the drug recovery percentages were calculated against the reference solution.

3. RESULTS AND DISCUSSION

3.1. LC method optimization

A simple RP-LC method was developed to assay dronedarone in pharmaceutical formulation. For this purpose, some parameters were studied including the use of different types of C18 column, solvent selection, composition and pH of the mobile phase. The proposed methodology was optimized to obtain a stability-indicating method.

Preliminary separations were tested using two different brands of reversed phase C_{18} column, Phenomenex Luna C18 (250 mm × 4.6 mm, 5 µm) and Waters XBridge™ C_{18} (250 mm × 4.6 mm, 5 µm). Waters XBridge™ C_{18} column provided the best chromatographic separation (acceptable theoretical plates and resolution) with symmetrical peak shapes (peak tailing factor < 2) combined with shorter analysis time.

Several mixtures were tested as mobile phase. First, buffer solution pH 4.9/ methanol/ acetonitrile mixture in different combinations was tested. The use of these different conditions achieved reproducible separations with an acceptable peak shape, although they showed peaks with poor resolution between dronedarone and its degradation products and prolonged the analysis time. To improve the resolution among degradation products, a mobile phase consisted of buffer solution pH 4.9 / acetonitrile was used. The separation of all components and retention time improved and therefore acetonitrile was selected as organic modifier. The effect of percent acetonitrile (ranging from 60 to 80% in the mobile phase) on the retention parameters was also investigated. It was concluded that 65% acetonitrile in the mobile phase resulted in better resolution of dronedarone and degradation products, improving the tailing factor and the retention time, with shorter analysis time.

The pH of the buffer solution (0.3% glacial acetic acid adjusted with TEA) was studied over a range from 3.8 to 6.0. The effects of pH on the chromatographic performance revealed that pH 4.9 was the most suitable due to a better tailing factor. It was found that the retention time of dronedarone, theoretical plates and tailing factor showed no significant changes at pH 3.8-4.9. However, adjusting the pH to higher values resulted in broad peaks and peak tailing.

The dronedarone UV spectra in the mobile phase were obtained from 190 to 400 nm using PDA detector. Dronedarone shows a maximum wavelength at 217 nm, although there is interference from the mobile phase composition in this low wavelength. Thus, the optimal wavelength was established at 289 nm.

Based on these investigations, good chromatographic dronedarone separation in the presence of its degradation products was achieved by using Waters XBridge™ C_{18} column and a simple mobile phase consisting of buffer solution pH 4.9 (0.3% glacial acetic acid adjusted with TEA): acetonitrile (35:65, v/v). These optimized conditions were validated for the analysis of dronedarone in tablets and resulted in a relatively short retention time of 5.2 min, allowing fast drug determination, which is important for routine analysis.

3.2. Forced degradation studies

Forced degradation studies were performed in order to provide the stability-indicating capability of the RP-LC method and the dronedarone degradation behavior since there is no available information about its susceptibility under stress conditions. Chromatograms of degraded samples are shown in Figure 2.

3.2.1. Hydrolytic conditions

Acid conditions

Acid degradation studies were initially performed in 1.0 M HCl maintained at 60°C for 6 h, although the peak area decreased only 2%. The reaction in 3.0 M HCl for 7 h at 80°C it was observed that almost 7.5% of the drug was degraded. The drug degradation resulted in an additional small peak at retention time of 4.11 min, which was well resolved from the dronedarone peak, as seen in the chromatogram shown in Fig. 2(c).

Basic conditions

The alkaline degraded dronedarone samples showed complete drug degradation in 1.0 M NaOH at 80°C for 3h. After 30 min of basic hydrolysis, around 60% of drug degradation was observed in 1.0 M NaOH and showed an additional peak at retention time of 2.8 min (Fig. 2d).

3.2.2. Oxidative conditions

Dronedarone was found to be stable in 3% H_2O_2 at room temperature for 24 h. However, the reaction of dronedarone with 30% H_2O_2 at room temperature for 48 h provided around 22% drug degradation. A compound was eluted before dronedarone using the RP-LC method. This degradation product peak was detected at retention time of 4.42 min (Fig. 2e). The resolution factor between the dronedarone peak and the degradation product peak was > 2.

3.2.3. Photolytic conditions

No significant photodegradation of dronedarone sample solution was observed after exposure to UV-A light (352 nm) for 8 h. On the other hand, exposing the drug to UV-A light at room temperature for 24h, around 26% degradation was observed. Almost 23% drug degradation was observed on exposure to UV-C light (254 nm) at room temperature for 1h30 min. No degradation peak was detected in any of the photodegraded samples, possibly indicating that the drug was degraded to non-chromophoric compounds (Fig. 2f and 2g).

3.2.4. Thermal conditions

In neutral thermal conditions, dronedarone exhibited a mild decrease of area and did not show any additional peaks (Fig. 2h).

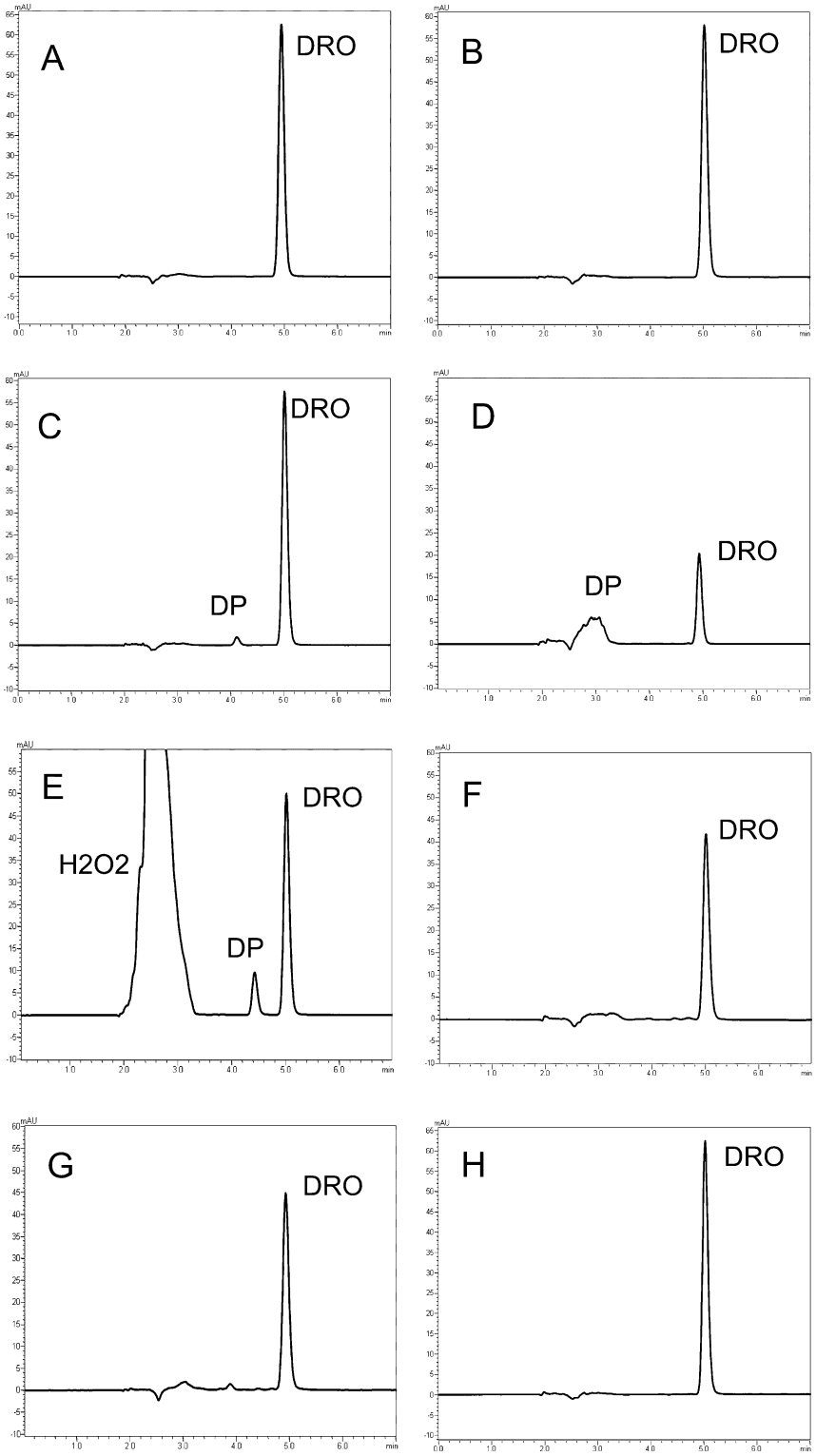

Figure 2. Chromatograms of dronedarone. (A) Dronedarone reference solution at 10 µg mL^{-1}. (B) Dronedarone tablet solution and chromatograms of degraded dronedarone in (C) acid hydrolysis in 3.0 M HCl at 80°C for 7 h; (D) basic hydrolysis in 1.0 M NaOH at 80°C for 30 min; (E) oxidative condition in 30% H_2O_2 for 48 h; (F) photolytic degradation in UVA for 24 h; (G) photolytic degradation in UVC for 1h30 and (H) thermal degradation at 80°C for 6 h. Chromatographic conditions: Waters XBridge C18 column (250 mm × 4.6 mm, 5 µm); mobile phase: buffer solution (0.3% glacial acetic acid, pH 4.9): acetonitrile (35:65, v/v); flow rate: 1.0 mL min^{-1}; detection: 289 nm.

3.5. Kinetics of dronedarone degradation under alkaline conditions

In order to determine the kinetic order of alkaline degradation, dronedarone solutions were also degraded in 1.0 M NaOH at 60°C. The kinetics data of dronedarone degradation under basic conditions indicated that the reaction follows an apparent first-order kinetics, which presented a linear relationship according to equation lnC = ln Co – kt, where C is the remaining DRO concentration, Co is the initial DRO concentration, k represents the slope and t is the time (h).The degradation rate constant (k) value was obtained by linear regression analysis and was found to be 2.33 x 10^{-1} µg ml^{-1} h^{-1} (y = -0.2327x + 2.9915, *r* = 0.9995), which was equal to the slope of the line taken with the opposite sign (Fig.3). The half-life ($t_{½}$) and t_{90} (time where 10% of initial drug concentration is degraded) were calculated and were found to be 3.0 h and 4.5× 10^{-2} h, respectively.

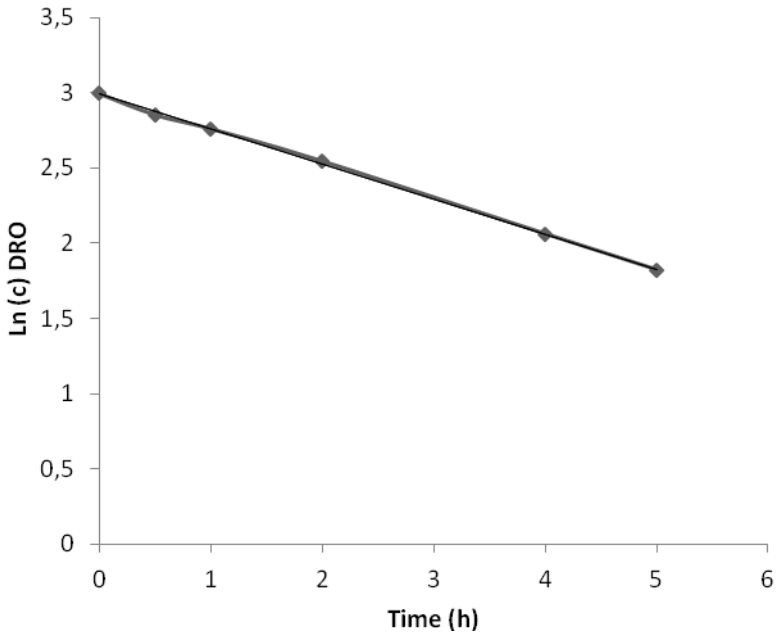

Figure 3**.** Kinetics plot of the alkaline degradation for dronedarone at 20 µg ml^{-1} using 1N NaOH at 60°C.

3.6. Validation of the RP-LC method

3.6.1. Specificity

The specificity of the RP-LC method was evaluated by the analysis of placebo and solutions containing the degradation products, which were compared with solution containing only dronedarone. In order to confirm the specificity of the method, dronedarone peaks generated by stress conditions were evaluated for peak purity using the PDA detector.

Analyzing the chromatogram shown in Figure 4, it could be observed that the placebo sample and the degradation products did not interfere with the dronedarone peak. In addition, the resolution factor between the dronedarone peak and the nearest resolving peak was $R > 2$, indicating well separated peaks. PDA analysis showed that no impurities and/or formulation excipients were co-eluting with the dronedarone peak, with peak purity index values higher than 0.9998, even in the solutions of stressed samples. Thus, these results demonstrated the ability of the RP-LC method to measure dronedarone in the presence of interferences.

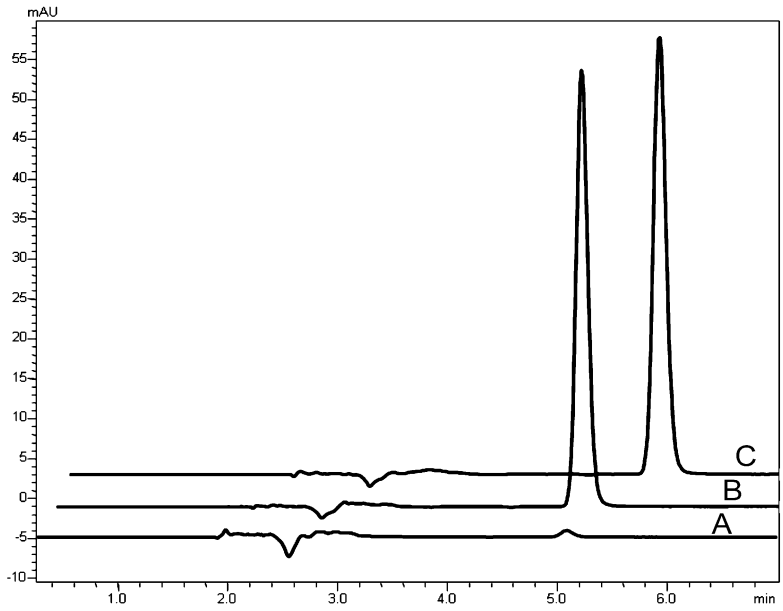

Figure 4. Chromatograms of placebo solution (A); dronedarone (20 µg mL^{-1}) sample solution (B) and reference solution (C). Chromatographic conditions: Waters XBridge C18 column (250 mm × 4.6 mm, 5 µm); mobile phase: buffer solution (0.3% glacial acetic acid, pH 4.9): acetonitrile (35:65, v/v); flow rate: 1.0 mL min^{-1}; detection: 289 nm.

3.6.2. Linearity and range

The three analytical curves for dronedarone were constructed by plotting concentration versus peak area. Linearity was obtained in the range from 5 to 100 µg ml^{-1}, which corresponds to 25 – 500 % of the intended test concentration of 20 µg ml^{-1}.The linear equation obtained by the method of least squares was y = 44111.42x + 21073.45, where x is the concentration and y is the absolute peak area. The correlation coefficient calculated (r = 0.9999) indicated good correlation between dronedarone concentration and peak area. The analytical data were validated by means of ANOVA, showing significant linear regression ($p > 0.05$) and non-significant linearity deviation ($p < 0.05$).

3.6.3. Precision

The repeatability of the RP-LC method was determined by calculating the relative standard deviation (RSD) of peak areas of six sample preparations at 20 µg ml^{-1} which resulted in RSD value less than 0.69% (n = 6). The intermediate precision was evaluated by analyzing six samples at three different days (inter-day) and by a second analyst (between-analysts). The precision results obtained are shown in Table 1. All RSD values were lower than 1.0%, confirming the excellent precision of the RP-LC method.

Table 1. Intra-day and inter-day precision data of RP-LC method for dronedarone.

Theoretical concentration (µg mL^{-1})	Intra-day			Inter-day		Between-analysts		
	Day	Assay [a] (%)	RSD (%)	Mean assay[b] (%)	RSD[b] (%)	Analyst	Assay [a] (%)	RSD[c] (%)
20	1	101.76	0.63	101.42	0.61	A	101.54	0.55
20	2	100.96	0.69			B	101.38	
20	3	101.54	0.14					

[a] Mean of six replicates.
[b] (n = 18)
[c] Relative standard deviation between Analyst A and B.

3.6.4. Accuracy

Accuracy was evaluated by assaying three different samples with known concentration to which known amounts of reference solution have been added, equivalent to 50, 100 and 150% of the nominal analytical concentration.

Concerning the accuracy evaluation, the mean recovery for the three concentration levels was found to be 99.30% (RSD = 0.59). The % recovery values are within the desired range (Table 2). According to results, the RP-LC method developed enables an accurate determination of dronedarone in pharmaceutical formulation.

Table 2. Recovery studies for the RP-LC method.

Nominal concentration (µg mL^{-1})	Added (µg mL^{-1})	Recovered ± SD (µg mL^{-1})[a]	Recovery (%)[a]	RSD (%)
10	5	4.97 ± 0.03	99.41	0.73
20	15	14.83 ± 0.08	98.90	0.56
30	25	24.90 ± 0.09	99.58	0.40

[a]Mean of three determinations for each concentration.

3.6.5. LOD and LOQ

LOD and LOQ of the RP-LC method were determined by using the mean of the slope, 44111.42 ± 160.63 and the standard deviation of the intercept of three independent curves, determined by a linear regression line as 4260.02. LOD and LOQ were calculated according to ICH guidelines (2005) and the theoretical values were 0.32 and 0.96 µg ml^{-1}, respectively. Experimentally, LOD was determined at the signal-to-noise ratio (S/N) of 3:1 and it was

found to be 1.0 µg ml^{-1}. LOQ was determined at S/N ratio of 10:1 with precision lower than 2%; results showed that LOQ was found to be 5.0 µg ml^{-1}.

3.6.6. Robustness studies

The robustness of the method is its ability to remain unaffected by small deliberate variations in chromatographic conditions. Robustness was assessed by analyzing the same sample solution under variations in the flow rate, acetonitrile ratio and pH value in the buffer solution. Results of robustness studies are shown in Table 3. Chromatographic responses were analyzed in each experiment. No significant changes were observed in theoretical plates and tailing factor, however retention time was between 4.21 and 6.47 min.

Variations in the chromatographic conditions did not show significant effect on the dronedarone percentages of commercial tablets (relative to their label claim concentration) obtained by comparing with the reference solution, thus demonstrating that the RP-LC method was robust.

The stability of reference and sample solutions was tested during 12 h and 24 h being stored at room temperature. The results showed that the retention time and assay values remained almost unchanged and no degradation was observed after 12h, suggesting that the sample was stable within the period indicated. However, after 24 h stored at room temperature, the % RSD of the dronedarone assay was 2.57%, relative to a freshly prepared reference solution, indicating that the sample was not stable up to 24 h when stored at room temperature.

Table 3. The robustness testing of the RP-LC method.

Experiment	Acetonitrile (%)	pH buffer	Flow rate (mL min $^{-1}$)	Assay (%)	T_f [a]	N [b]	t_R [c]
1	60	4.9	1.0	100.76	1.17	8648	6.02
2 [d]	65	4.9	1.0	101.49	1.21	9154	5.02
3	70	4.9	1.0	100.18	1.23	7670	4.30
4	65	4.4	1.0	101.18	1.24	8219	5.31
5	65	5.4	1.0	101.17	1.14	10552	6.47
6	65	4.9	0.8	101.27	1.21	9608	6.24
7	65	4.9	1.2	99.54	1.21	7534	4.21

[a] Tailing factor.
[b] Theoretical plates.
[c] Retention time.
[d] Optimal conditions.

3.6.7. System suitability

The system suitability test was carried out according to USP 34 [13] by determining the capacity factor (k'), theoretical plates (N) and tailing factors (T_f). The capacity factor was found to be 2.34 (RSD = 0.11%). The number of theoretical plates was 9154.56 (RSD = 1.21%) and tailing factors were 1.21 (RSD = 0.17%). The results for peak area showed RSD values of 0.45%. System suitability results were within the acceptable values (RSD < 2.0%) indicating that the chromatographic system was adequate for the analysis intended.

3.7. Application of the RP-LC method to pharmaceutical analysis

The validated RP-LC method was applied to the assay of dronedarone in commercial pharmaceutical formulation. The mean percentage found (Table 1) was in agreement with the

claimed label, indicating that the method proposed could be adopted for quality control analysis of dronedarone.

3.8. UV optimization method

The UV method for the assay of dronedarone was developed as an alternative technique, considering its rapid and low-cost quantitation of dronedarone, and its simple instrumentation compared with other techniques. Since dronedarone is soluble in methanol this solvent was chosen for the UV assay.

3.9. Validation of the UV method

3.9.1. Specificity

In order to verify the specificity of the spectrophotometric method, the UV spectrum of the placebo solution was obtained and compared with the spectra of reference and sample solutions. As can be seen in Figure 5, there was no interference from the tablet excipients in the maximum dronedarone absorption in 289 nm, thus demonstrating that the method is specific.

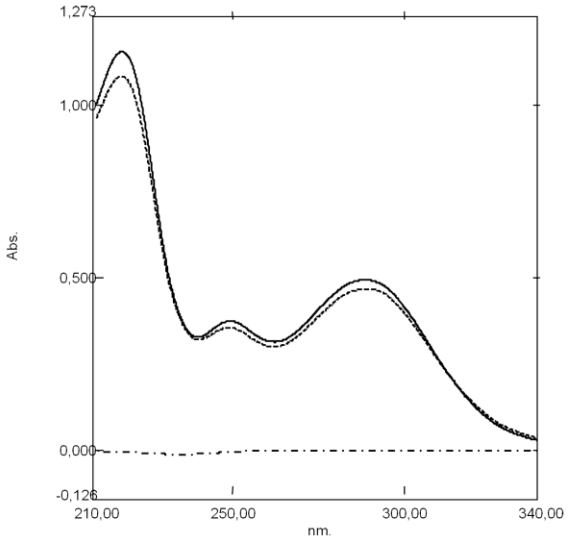

Figure 5**.** UV spectra of dronedarone (15.0 µg ml^{-1}) reference solution (--), sample solution (B) (–) and placebo solution (-·) in methanol.

3.9.2. Linearity and range

To assess the linearity of the spectrophotometric method, three independent analytical curves for dronedarone were constructed. Good linearity was observed in the concentration range from 5.0 to 25.0 µg ml^{-1}. The representative linear equation was y = 0.0326 + 0.0144, obtained by the method of least squares, showing adequate correlation coefficient (r = 0.9999). Data were validated by means of ANOVA, which demonstrated significant linear regression ($p > 0.05$) with non-significant linearity deviation ($p < 0.05$).

3.9.3. Precision

The precision of the spectrophotometric method was assessed by the repeatability and intermediate precision. Repeatability was expressed as the RSD value obtained by using six sample determinations at 15 µg ml^{-1}. The intermediate precision analysis was carried out by comparing assays in six determinations at 15 µg ml^{-1} on three different days and between-analysts. The RSD values were found to be less than 1%. The results of repeatability and intermediate precision were acceptable, demonstrating adequate precision of the UV method, as shown in Table 4.

Table 4. Intra-day and inter-day precision data of UV method for dronedarone.

	Intra-day			Inter-day		Between-analysts		
Theoretical concentration (µg mL^{-1})	Day	Assay[a] (%)	RSD (%)	Mean assay[b] (%)	RSD[b] (%)	Analyst	Assay[a] (%)	RSD[c] (%)
15	1	100.67	0.53			A	100.84	
15	2	100.88	0.61	100.80	0.59	B	100.77	0.66
15	3	100.84	0.77					

[a]Mean of six replicates.
[b] (n = 18)
[c] (n = 12)

3.9.4. Accuracy

Accuracy was assessed by determination of dronedarone in solution by the standard addition method. The assay was carried out by adding known amounts of reference solution, corresponding to three concentration levels, in a sample solution with known concentration. Regarding the accuracy of the spectrophotometric method, the results are similar to those obtained by the LC method. All recoveries of dronedarone reference standard were in the range from 98.0 to 102.0%, as shown in Table 5.

Table 5. Recovery studies for the UV method.

Nominal concentration ($\mu g\ mL^{-1}$)	Added ($\mu g\ mL^{-1}$)	Recovered ± SD ($\mu g\ mL^{-1}$)[a]	Recovery (%)[a]	RSD (%)
10	5	5.02 ± 0.07	100.44	1.42
15	10	10.05 ± 0.15	100.50	1.51
20	15	15.03 ± 0.15	100.26	1.03

[a]Mean of three determinations for each concentration.

3.9.5. LOD and LOQ

The limit of detection and limit of quantitation were calculated based on the standard deviation of the y-intercepts and the slope of three analytical curves. LOD and LOQ of the UV method were 0.44 and 1.32 $\mu g\ ml^{-1}$, respectively. Although LOD and LOQ of the UV method were higher than those obtained for the LC method using the same approach, they are adequate to assay dronedarone in the range proposed in the UV method.

3.9.6. Robustness

The robustness of the UV method was evaluated by analyzing reference and sample solutions at 15 µg ml^{-1} using different wavelengths (287, 289 and 291 nm) and two different methanol brands. There were non-significant changes in the results using different wavelengths and methanol brands, showing that the method is robust.

3.10. Comparison between the RP-LC and UV methods

RP-LC and UV methods mused to assay dronedarone in pharmaceutical formulation, proposed in our study, were compared using statistical analysis by Student's t-test. There was non-significant difference between experimental assay values obtained through the analysis of twelve solutions of pharmaceutical dosage form using both methods ($p > 0.05$) (Table 6).

Table 6. Statistical comparison of the results obtained by the RP-LC and UV methods.

Parameters	RP-LC	UV
Mean assay (%)	101.32	101.13
SD	0.1855	0.4406
RSD	0.1830	0.4356
n	12	12
Variance	0.034	0.1941
Student's *t*-test	1.351 (2.074)[a]	

[a] Figure between the parenthesis represents the corresponding tabulated value of t at $p = 0.05$.

4. Conclusion

The validation results obtained by the RP-LC method are in good agreement with those obtained by the UV spectrophotometric method. Both methods are specific, accurate, linear, precise and robust, without any interference of tablet excipients, allowing complete interchange. The proposed RP-LC method has ability to separate dronedarone from its degradation products. The forced dronedarone degradation behavior in pharmaceutical dosage form was examined for the first time in this study under RP-LC conditions. The kinetics of the alkaline dronedarone degradation was studied in 1.0 M NaOH at 60°C and found to be first-order reaction. The methods proposed provided simple and rapid dronedarone determinations in film-coated tablets and could be applied in the quality control of pharmaceutical dosage forms and stability studies.

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ARTIGO 2 – DETERMINAÇÃO DE DRONEDARONA EM FORMA FARMACÊUTICA POR CROMATOGRAFIA ELETROCINÉTICA MICELAR

5 ARTIGO 2 – DETERMINAÇÃO DE DRONADARONA EM FORMA FARMACÊUTICA POR CROMATOGRAFIA ELETROCINÉTICA MICELAR

Publicação científica: Determination of dronedarone in pharmaceutical dosage form by stability-indicating micellar electrokinetic chromatography method.

Determination of Dronedarone in Pharmaceutical Dosage Form by Stability-Indicating Micellar Electrokinetic Chromatography Method

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Abstract:

In this study, a micellar electrokinetic chromatography method was developed and validated for the analysis of dronedarone in film-coated tablets. Electrophoretic conditions were investigated by changing factors such as pH, buffer concentration, SDS concentration, capillary temperature, injection time and applied voltage. Separation was performed using a bare fused-silica capillary of 40.0 cm effective length (48.5 cm total length; 50 µm internal diameter) maintained at 30°C and detection was at 216 nm. Optimal conditions were obtained using 40 mM borate buffer and 50 mM SDS at pH 9.2 as background electrolyte with an applied voltage of 28 kV (positive polarity) and using hydrodynamic injection at 50 mbar for 7 s. The method was validated evaluating typical validation characteristics such as specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. The analytical curve was linear in the concentration range from 25 to 150 µg/mL (r = 0.9995). The accuracy was 99.9% and the relative standard deviations of repeatability and intermediate precision were lower than 2%. The limit of detection and limit of quantitation were 0.88 µg/mL and 2.66 µg/mL, respectively. The method proved to be robust by a fractional factorial design evaluation. Forced degradation studies were performed by exposing dronedarone sample solution to stress conditions (acidic, basic, oxidative, thermal and photolytic) in order to verify the stability-indicating capability of the method. The MEKC method was successfully applied for the quality control of dronedarone hydrochloride in commercially film-coated tablets.

Keywords: Dronedarone. Micellar electrokinetic chromatography. Stability-indicating method. Validation.

1. Introduction

Dronedarone HCl (DRO; SR33589B; Fig. 1) is a noniodinated benzofuran derivative developed as an antiarrhythmic agent for the management of atrial fibrillation (AF) (SUN et al., 1999; SUN et al., 2002; GAUTIER et al., 2003; TOUBOUL et al., 2003; FDA, 2009). AF is the most common cardiac arrhythmia in elderly patients, associated with an increased risk of stroke and mortality and therefore, is responsible for high medical costs (GO et al., 2001; NATTEL; 2002).

Amiodarone is one of the most effective prescribed antiarrhythmic drug for the treatment of AF, although it is associated with numerous side effects such as thyroid dysfunction, pulmonary fibrosis, gastrointestinal, neurological and dermatological disorders (GOLDSTEIN; STAMBLER, 2005). DRO is structurally related to amiodarone and was designed to overcome the side effects of its parent compound. The removal of the iodine moiety was intended to minimize the toxic effects on the thyroid and other organs. The addition of a methylsulfonamide group was aimed to decrease lipophilicity and, thus, shortening the half-life and the potential neurotoxic adverse events (SUN et al., 1999; SUN et al., 2002; WEGENER et al., 2006; ZIMETBAUM et al., 2009). The cardiac electrophysiological effects of DRO are similar to those amiodarone (VARRÓ et al., 2001). This novel antiarrhythmic drug has been shown to prevent the recurrence of AF by slowing the ventricular rate during arrhythmia. Thus, DRO is associated with the reduction in the incidence of hospitalization due to heart failure in patients with AF (TOUBOUL et al., 2003; SINGH et al., 2007; HOHNLOSER et al., 2009).

Pharmaceutical analysis is usually performed by high-performance liquid chromatography (HPLC), which is the standard method in many industries (ALTRIA, 1999). However, in the last years, capillary electrophoresis (CE) has been used as an alternative for HPLC due to its numerous advantages such as high separation efficiency, analysis speed, instrument simplicity, reduced method development time, reduced operating costs and solvent consumption (ALTRIA, 1999; NISHI, 1999).

Micellar electrokinetic chromatography (MEKC) is a type of chromatography based on micellar solubilization and electrokinetic migration, performed by dissolving an ionic surfactant in the CE solution at a concentration higher than the critical micelle concentration. The separation mechanism is based on partitioning of the analyte between the micelle and the running buffer, hence the micelles are called a pseudostationary phase, corresponding to the stationary phase in conventional chromatography. MEKC is a powerful separation mode of

CE without instrumental modifications, used for the separation of small neutral molecules such as water-insoluble compounds, which cannot be separated by capillary zone electrophoresis (CZE) (TERABE et al., 1984; OTSUKA et al., 1985; TERABE et al., 1985; KIM et al., 2001; TERABE, 2004; TERABE, 2010).

A survey of the literature revealed that no capillary electrophoresis method is available for the determination of DRO in pharmaceutical formulation. Therefore, the aim of this work was to develop and validate a MEKC method in accordance with the ICH guidelines as an alternative tool for the analysis of DRO in film-coated tablets, considering the ever increasing demand of pharmaceutical research.

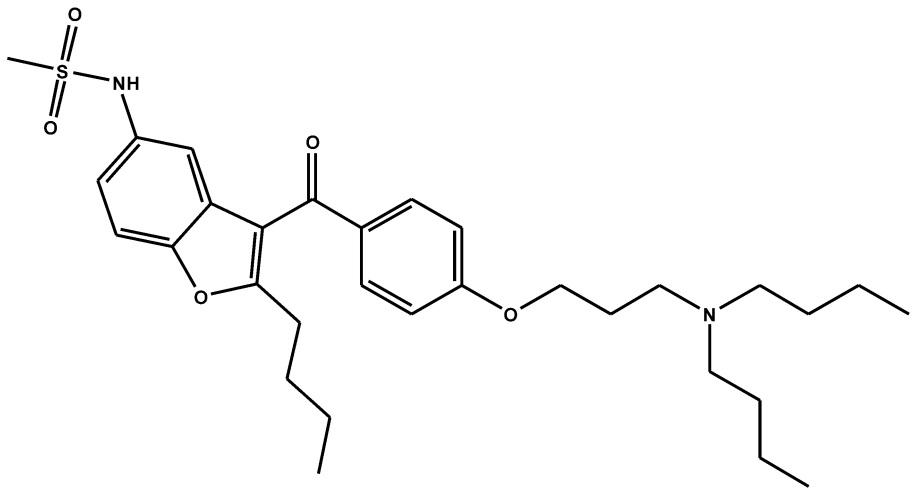

Figure 1. Chemical structure of dronedarone.

2. Experimental

2.1. Chemicals

Dronedarone hydrochloride reference standard (assigned purity 99.7%) was purchased from Sequoia Research Products (Pangbourne, Berkshire, UK) and nimesulide reference substance, used as internal standard, was supplied by Brazilian Pharmacopoeia (Rio de Janeiro, Brazil). Multaq® (Sanofi-Aventis, Paris, France) film-coated tablets, containing 400 mg of dronedarone hydrochloride were obtained from commercial sources. Boric acid was obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol was purchased

from Tedia Company Inc (Fairfield, OH, USA). Hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were purchased from Merck KGaA (Darmstadt, Germany). Sodium acetate, sodium dodecyl sulphate (SDS) and sodium hydroxide (NaOH) were obtained from Synth (Diadema, SP, Brazil). Ultrapure water from a Milli-Q water purification system (Milli Q Gradient System, Millipore Corp., Bedford, MA, USA) was used for all analysis.

2.2. Instrumentation

Analyses were conducted on an Agilent ^{3D}CE apparatus (Agilent Technologies, Waldbronn, Germany). This unit was equipped with an autosampler, a photodiode array (PDA) detector and power supply able to deliver up to 30 kV. The CE ChemStation software was used to perform data acquisition, data handling and for instrument control.

2.3. Background electrolyte preparation

The background electrolyte (BGE) solution was obtained by separately dissolving the required amount of boric acid and SDS in ultrapure water in order to obtain 100 mM stock solutions. The BGE solution containing 40 mM borate buffer and 50 mM of SDS was daily prepared by homogenizing the required volumes of the stock solutions and diluting in ultrapure water. The pH value was adjusted to 9.2 with 1 M NaOH. This solution was filtered through a 0.22 µm Millex (Millipore Corp.) filter.

2.4. Preparation of reference solution

DRO stock solution containing 1 mg/mL was prepared by weighting 10 mg of reference standard into 10 mL volumetric flask and dissolving with methanol. This solution was stored at 2-8°C. Working solutions were daily prepared by diluting appropriate volume of the stock solution with BGE solution. Stock solution of nimesulide (0.7 mg/mL) was prepared in methanol.

2.5. Preparation of sample solution

The samples were daily prepared by accurately weighting an amount of the powder equivalent to 25 mg of DRO, obtained by crushing and homogenizing twenty film-coated

tablets, each containing 400 mg of dronedarone. This amount of powder was transferred into 25 mL volumetric flask with 20 mL of methanol and, then, the samples were sonicated for 15 min. After, the volume of flasks was filled up with methanol to obtain concentration of 1 mg/mL. An aliquot of this solution was diluted with BGE to an appropriate concentration and filtered through 0.45 µm membrane filter (Millipore Corp) for analysis.

2.6. Electrophoretic procedure

All separations were carried out on a bare fused-silica capillary of 40 cm of effective length (48.5 cm total length) and 50 µm internal diameter (i.d.), thermostatized at 30°C. Prior to the first use, the new capillary was conditioned by flushing with 1 M NaOH during 30 min and with ultrapure water during 15 min. Every morning, previously starting analysis, the capillary was sequentially rinsed by with 0.1 M NaOH for 20 min, ultrapure water for 15 min and finally with the BGE solution for 20 min. Before each sample injection, the capillary was preconditioned with 0.1 M NaOH for 4 min, ultrapure water for 2 min and the BGE solution for 2 min in order to obtain migration time reproducibility. The following optimized conditions were applied for sample injection: hydrodynamic injection at 50 mbar for 7 s applying a voltage of 28 kV (current was between 50 and 70 µA). PDA detection was monitored at 216 nm. The BGE solution in capillary inlet and outlet vials was replaced by a refresh solution every three runs to prevent changes in the migration time.

2.7. Statistical software

The experimental designs and statistical analysis of data were carried out by the MINITAB 14 (Minitab Inc, State College, PA, USA) data analysis software system.

2.8. Validation of the MEKC method

The MEKC method was intended to quantitative measure DRO in film-coated tablets using nimesulide as internal standard (IS). Validation was performed according to ICH guidelines (2005) by evaluating the following characteristics: specificity, linearity and range, precision, accuracy, limit of detection, limit of quantitation, robustness. System suitability test was carried out according to USP 34 (2011).

2.8.1. Specificity and forced degradation studies

Specificity and forced degradation studies were performed by exposing a tablet solution, containing 1 mg/mL, under different stress conditions (acidic and basic hydrolysis, heat, oxidation and UV light):

a) Hydrolytic conditions: Sample solutions were treated with 0.1 M HCl during 3 h for acidic hydrolysis and with 0.5 M NaOH during 6 h for basic hydrolysis, both maintained at ambient temperature. Then, solutions were neutralized with base or acid, as necessary, and diluted with the BGE solution at a concentration of DRO of 50 µg/mL.

b) Oxidative conditions: These studies were performed by storing the tablet solution in 25% H_2O_2 for 5 h, protected from the light, at ambient temperature.

c) Dry heat degradation: For thermal neutral degradation, tablet solution (1 mg/mL) was diluted in water and placed in the oven at 45°C for 8 h.

d) Light testing: Photolytic degradation was carried out with tablet solutions, which were exposed to near UVC light (254 nm) in a photostability chamber for 5 h, side-by-side with a protected sample used as dark control. Samples were withdrawn at the end of the exposure period.

The tablet solutions stored under normal conditions and the zero time stressed samples were used as controls for comparison with the stressed samples, in order to characterize the peaks assigned to stress agents. Prior to MEKC analysis, nimesulide was added as IS and samples were diluted with BGE solution to obtain final concentrations of 50 µg/mL and 35 µg/mL for dronedarone and nimesulide, respectively.

The specificity of the method was also studied by preparing a placebo (in-house mixture of all tablet excipients) and by determining the peak purity of stressed samples using a PDA detector, in order to verify the interference from the tablet excipients and degradation products.

2.8.2. Linearity and range

Linearity of the MEKC method was determined by injecting five standard concentrations of DRO in the range from 25 to 150 µg/mL (25; 50; 75; 100 and 150 µg/mL). The reference solutions were spiked with nimesulide (IS) and diluted with the BGE solution (IS final concentration of 35 µg/mL). Three independent analytical curves were constructed by plotting the peak area ratio against the respective DRO concentration for the establishment of linearity, which was evaluated by the method of least squares.

2.8.3. Accuracy

In order to determine the accuracy of the MEKC method, a recovery study was performed in triplicate. Know amounts of DRO reference substance were added to a sample solution (containing 25 µg/mL of DRO), to obtain three concentration levels (40, 50 and 60 µg/mL) corresponding to 80, 100 and 120% of the test concentration. Accuracy was calculated as the percent recoveries of the DRO reference substance.

2.8.4. Precision

The precision of the method was assessed at two levels: repeatability and intermediate precision. Repeatability was investigated by analysis of six sample solutions at same nominal concentration of DRO (50 µg/mL) spiked with IS (35 µg/mL), on the same day and under the same operating conditions. The analysis of six sample solutions at 50 µg/mL was performed on different days in order to determine the intermediate precision. The precision between peak area ratio measurements was expressed as relative standard deviation (RSD).

2.8.5. Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined from the standard deviation of y- intercepts (σ) and the slope (S) obtained from three regression lines and were calculated using the equations defined by ICH guidelines (2005): $LOD = 3.3(\sigma/S)$ and $LOQ = 10 (\sigma/S)$.

2.8.6. Robustness

The robustness test was evaluated by using an experimental design procedure. The procedure selected was a 2-Level 2^{4-1} (eight experiments) fractional factorial design performed by the selection of four factors: pH borate buffer, borate buffer concentration, SDS buffer concentration and voltage. All the factors were studied at two levels: high and low. The factors and their levels evaluated in this study are presented in Table 1. The obtained response (assay; %) was processed by Minitab® 14 statistical software (Minitab Inc, State College, PA, USA) to evaluate the significance of the effects.

Table 1**.** Variables selected as factors and values chosen as levels to evaluate the robustness of the method.

Factors	Optimal	Low level	High level
pH borate buffer	9.2	9.1	9.3
Borate buffer concentration (mM)	40	38	42
SDS buffer concentration (mM)	50	48	52
Voltage (kV)	28	27	29

2.8.7. Solution stability

The solution stability was carried out by leaving the sample solutions into instrument vials, which were placed in the autosampler for 12 h at room temperature.

2.8.8. System suitability test

System suitability test was carried out to ensure that the CE system, the MEKC method and samples constitute an integral system, which is reproducible and adequate for the analysis. The parameters such as column efficiency (theoretical plates), resolution, tailing factor, migration time and capacity factor were checked by injecting DRO reference solution (containing 50 µg/mL) in six replicates, in accordance to USP 34 (2011).

2.9. Assay of pharmaceutical formulation

The procedure mentioned in Section 2.5 was used for the analysis of DRO in five film-coated tablets. As DRO is practically insoluble in aqueous solutions, the sample solutions containing 1 mg/mL were diluted with the BGE solution to obtain a final concentration of 50 µg/mL. Then, these solutions were filtered through 0.45 µm membrane filter (Millipore Corp) and each one was injected in triplicate. The DRO concentrations were determined by calculating the DRO peak areas relative to those of an internal standard peaks. The peak area ratios obtained in the sample solutions were compared with those obtained for the reference solution.

3. Results and discussion

3.1. Development of the MEKC method

3.1.1. Effect of the running buffer pH

The effect of pH in MEKC is the most powerful parameter in optimizing the separation, since EOF depends on the pH because it determines the degree of ionization of the silanol group on the capillary surface, even though the electrophoretic velocity of the SDS micelle does not depend on the pH (TERABE, 2010). Ionization of the analytes affects micellar solubilization, hence the migration time depends on the pH for acid or basic analytes. In electrophoresis, only charged solutes are subject to electrophoretic migration; consequently, the solute must be ionized under electrophoretic conditions for a separation to occur (TERABE et al., 1988). A buffer system containing sodium acetate was tested in the pH value of 4.5, however no separation occurred and no peaks were observed. According to Terabe (1988), the optimum pH value to the separation of two compounds is always close to their pKa. Therefore, as DRO pKa is 9.4, an optimal separation of DRO was achieved by using borate buffer and SDS solution at the concentrations of 40 mM and 50 mM at the pH values from 8.8 to 9.2 (AusPAR, 2010). Higher pH values resulted in increase of migration time and peak width, but with excellent peak shape. Lower pH values resulted in peak broadening.

3.1.2. Effect of the buffer concentration

The effect of the concentration of borate buffer was studied in the concentration range of 10-50 mM with 50 mM SDS at a pH 8.8, using a temperature of 30°C and a voltage of 25 kV. The ionic strength of the borate affects the magnitude of the EOF. Using a higher ionic strength will result in a lower EOF and, consequently, the migration time increased. Experimentally, it was found that the migration time increases with increasing borate concentration. Hence, 40 mM borate buffer was chosen because it provided a good migration time with an adequate retention factor (k). Methanol was added at concentrations of 2-10% to the borate buffer in order to study the effects of organic solvents: the migration time was longer and no improvements were observed.

3.1.3. Effect of SDS concentration

SDS is the most commonly surfactant employed in MEKC, where SDS micelles are used as a pseudostationary phase and separations are based on the partitioning between the micelles and the buffer solution (OZAKI et al., 1995; TERABE, 2010). In order to form anionic micelles, SDS was used at a concentration higher than its critical micelle concentration (CMC) of 8 mM (TERABE, 2004). The effects of the concentrations of SDS were determined in the range of 25-70 mM. The retention factor is linearly proportional to the surfactant concentration; however, higher concentration of SDS raises the current in the capillary and increases the migration time (TERABE et al., 1985). Due to the facts mentioned above, 50mM was chosen as the optimum SDS concentration, considering appropriate separation, run time and internal temperature.

3.1.4. Effect of the capillary temperature

In order to achieve high reproducibility of the migration times, MEKC separation should be performed at a constant temperature. The effect of capillary temperature was investigated at 25, 30 and 35°C. Problems with joule heating could be observed at 35°C. Thus, the temperature of 30°C was selected due to adequate separation and reasonable run time with acceptable current.

3.1.5. Effect of the injection time

Low concentration sensitivity in CE is attributed to the reduced loadability and short optical path length for absorbance detectors. In order to increase concentration sensitivity, the samples solutions were hydrodynamically injected at 50 mbar while injection time was changed from 5 to 7 s. The injection time of 7 s was selected due to the best peak shape and peak area ratio reproducibility.

3.1.6. Effect of the applied voltage

The influence of the applied voltage was verified by changing the voltage from 23 to 29 kV under the optimized BGE conditions. Voltage of 28 kV was selected as the working voltage because it provided shorter migration times, best peak symmetry and good resolution between DRO and the degradation products peaks.

3.1.7. Optimized conditions

Considering the results of the electrophoretic parameters, MEKC optimized conditions were obtained with 40 mM borate buffer and 50 mM SDS at pH 9.2. The optimal wavelength was investigated in the range of 190-400 nm. Detection was set at 216 nm since this is the wavelength of maximum UV absorption for DRO. The samples were injected hydrodynamically for 7 s with applied voltage of at 28 kV. A suitable separation performance was achieved with the best peak area, symmetry, resolution and good migration times within a run time of 7.0 min.

3.2. Method validation

Validation of the MEKC method was performed in accordance to the ICH guidelines (2005). Nimesulide was used as an IS approach, which provided the easily identification of the analyte peak by its migration time and the calculation of the peak area ratio, thus improving the reproducibility of the method. Nimesulide was added to each sample solution at a concentration of 35 µg/mL.

3.2.1. Specificity and forced degradation studies

Prior to initiating the MEKC method development, forced degradation samples were used to assess the stability-indicating capacity of the separation method. Forced degradation in 0.1 M HCl showed a decrease of peak area of 13% and produced a new peak, which indicated that the drug was unstable under acidic hydrolysis. Under alkaline conditions, 17.4% of DRO degradation was seen. Moreover, and additional peak was detected at 4.5 min. The oxidative degradation resulted in decrease of peak area around 24% and produced an additional peak at 4.76 min. No degradation products were detected in photolytic or thermal conditions even though around 55% and 13% degradation was seen, respectively. This could be due to the poor sensitivity of the CE, relating to the small amount of sample injected and a short optical path length for absorbance detector (TERABE, 2004). The electropherograms of forced degradation studies were analyzed considering resolution between DRO peak and degradation products formed in stress conditions and, as shown in Fig. 2, all degradation products were separated from the DRO peak. Co-migrating peaks of degradation products were not observed, which was confirmed by the peak purity of DRO peak obtained using the PDA detector.

The specificity of the MEKC method was also determined by comparing the electropherograms of reference solution, tablet solution and placebo. From the electropherograms shown in Fig. 3, it was observed that placebo did not show any significant interference with the DRO peak since no new peak was detected during analysis. Therefore, it is important to consider that no interferences either from tablet excipients or peaks generated by forced degradation studies interfered in the determination of the DRO peak, demonstrating the specificity of the proposed MEKC method and its stability-indicating capability.

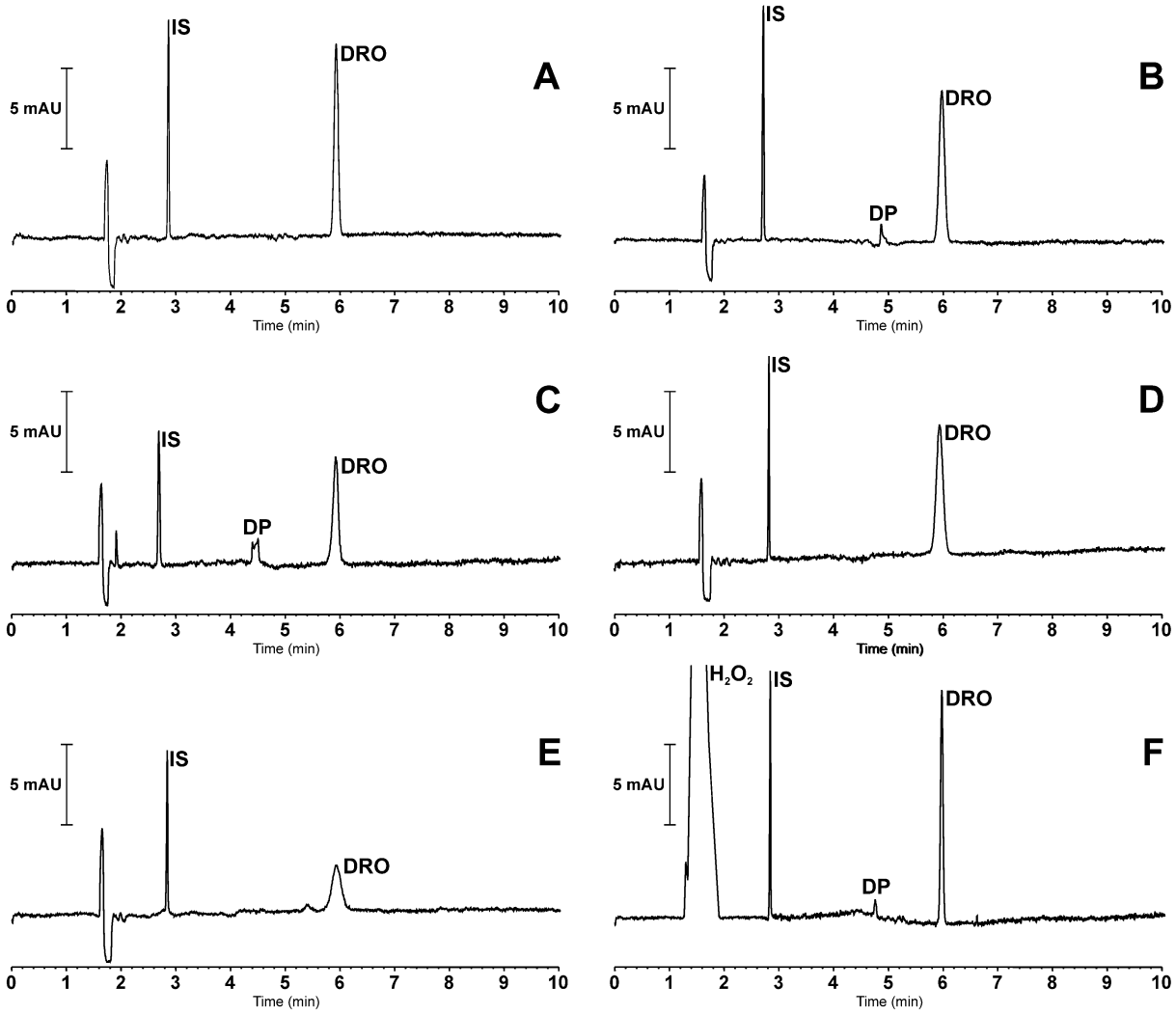

Figure 2. MEKC electropherograms of dronedarone (DRO; 50 µg/mL) and nimesulide (IS, 35µg/mL): DRO reference substance solution (A); DRO after acid hydrolysis (B); DRO after basic hydrolysis (C); DRO after thermal degradation (D); DRO after exposition to UVC light (E); and DRO after oxidation (F). Operating conditions: 40 mM borate buffer and 50 mM SDS, pH 9.2; voltage: 28 kV; capillary temperature: 30°C; detection: 216 nm. DP: degradation product; IS: internal standard.

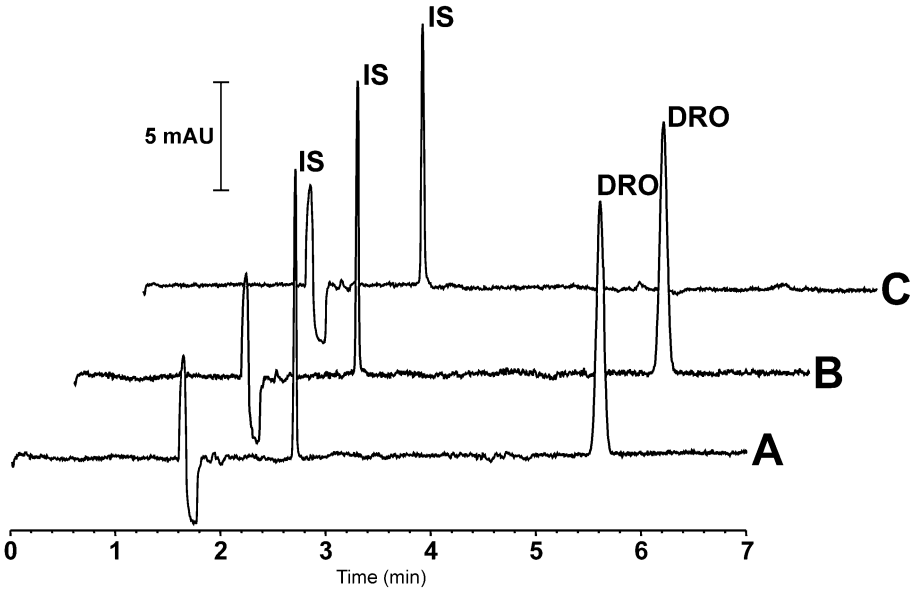

Figure 3**.** Typical electropherograms of dronedarone (DRO) containing nimesulide (IS; 35 µg/mL) obtained from the injection of DRO reference solution (50 µg/mL) (A); tablet solution (50 µg/mL) (B) and placebo solution (C). Operating conditions: 40 mM borate buffer and 50 mM SDS, pH 9.2; voltage: 28 kV; capillary temperature: 30°C; detection: 216 nm.

3.2.2. Linearity

The linear analytical curves for DRO were obtained in the concentration range from 25 to 150 µg/mL, corresponding to 50-300% of the test concentration (50 µg/mL). Linearity was assessed by means of least squares linear regression analysis. The regression equation obtained ($y = 1.0039x – 0.4684$, where x is the standard concentration of DRO in µg/mL and y is the peak area ratio of DRO to IS), with the calculated correlation coefficient of 0.9995, confirmed the linearity of the MEKC method over the concentration range. An analysis of variance (ANOVA) was carried out to evaluate the validity of the assay, which demonstrated significant linear regression ($p < 0.05$) and non-significant linearity deviation ($p > 0.05$).

3.2.3. Accuracy

The accuracy of the MEKC method was assessed by calculating percentage recovery of added DRO reference substance. Accuracy was performed using three concentration levels with three replicates each. The mean percent recovery for DRO was 99.9% (RSD = 1.09%; $n = 9$). The results were in agreement with the desired range of 100.0 ± 2% at each

concentration level over the range from 80 to 120% of the test concentration, as shown in Table 2 (SHABIR, 2003).

Table 2. Recovery studies for the MEKC method.

Nominal concentration (μg mL^{-1})	Added (μg mL^{-1})	Recovered ± SD (μg mL^{-1})[a]	Recovery (%)[a]	RSD (%)
40	15	15.05 ± 0.05	100.33	0.36
50	25	24.65 ± 0.03	98.61	0.12
60	35	35.34 ± 0.08	100.98	0.24

[a]Mean of three determinations for each concentration.

3.2.4. Precision

The precision was investigated in terms of repeatability and intermediate precision for DRO peak area ratio. To determine repeatability, six different samples with the same concentration were injected and analyzed. The relative standard deviation values of the assay results are shown in Table 3. Intermediate precision establishes the effects of random events on results obtained under within-lab typical variations, such as different days, analysts, equipments, etc. (SHABIR, 2003; ICH, 2005). Concerning intermediate precision, six samples were analyzed on different days and a RSD value less than 1% was obtained, demonstrating the excellent precision of the MEKC method.

Table 3. Repeatability (intra-day) and intermediate precision (inter-day) data of the MEKC method for dronedarone.

Theoretical concentration (µg mL $^{-1}$)	Intra-day			Inter-day	
	Day	Assay[a] (%)	RSD (%)	Mean assay[b] (%)	RSD (%)
50	1	101.09	0.55	100.82	0.65
50	2	100.56	0.67		

[a]Mean of six determinations for each day.
[b](n = 12).

3.2.5. Limit of detection and limit of quantification

LOD and LOQ were calculated from the slope and the standard deviation using the mean values of three regression lines. LOD was 0.88 µg/mL and LOQ was 2.66 µg/mL. These results were acceptable, due to the low sensitivity of the MEKC methods.

3.2.6. Robustness

In the determination of the method robustness, the significance of the effects was evaluated by a Pareto chart of the standardized effects and normal probability plot of the residuals. The codes A, B, C, and D correspond to the pH borate buffer, borate buffer concentration, SDS buffer concentration, and voltage, respectively. The combination of two codes indicates the interaction effect between the two variables. Pareto chart consists of bars with a length proportional to the absolute value of the estimated effect, divided by the pseudo standard error defined by Lenth (Lenth's PSE) (1989). The bars were displayed based in the size of the effect, with the largest effect on the top. The chart includes a vertical line at the critical *t*-value for an α of 0.05. Effects in which the bars are smaller than the critical *t*-value were not considered significant and did not affect the response variables. The effects of the factors, at the studied ranges, were not statistically significant (α = 0.05) for the assay, represented by Fig. 4(a). The normal probability plot of residuals consists of the difference between the predicted values (as predicted by the current model) and the observed values. Since all values fall onto a straight line it can be concluded that they follow the normal distribution (VANAJA; RANI, 2007). Figure 4(b) presents the normal probability plot of

residuals for DRO (residual values plotted along the horizontal X-axis; the vertical Y-axis shows the expected normal values for the respective values, after they are rank-ordered). At the studied ranges, the effects of the factors were not statistically significant ($p > 0.05$) for the response studied (assay). Therefore, there were no significant changes in the assay regarding the percentage of DRO contents under the modifications made in the experimental conditions, showing the robustness of the developed method.

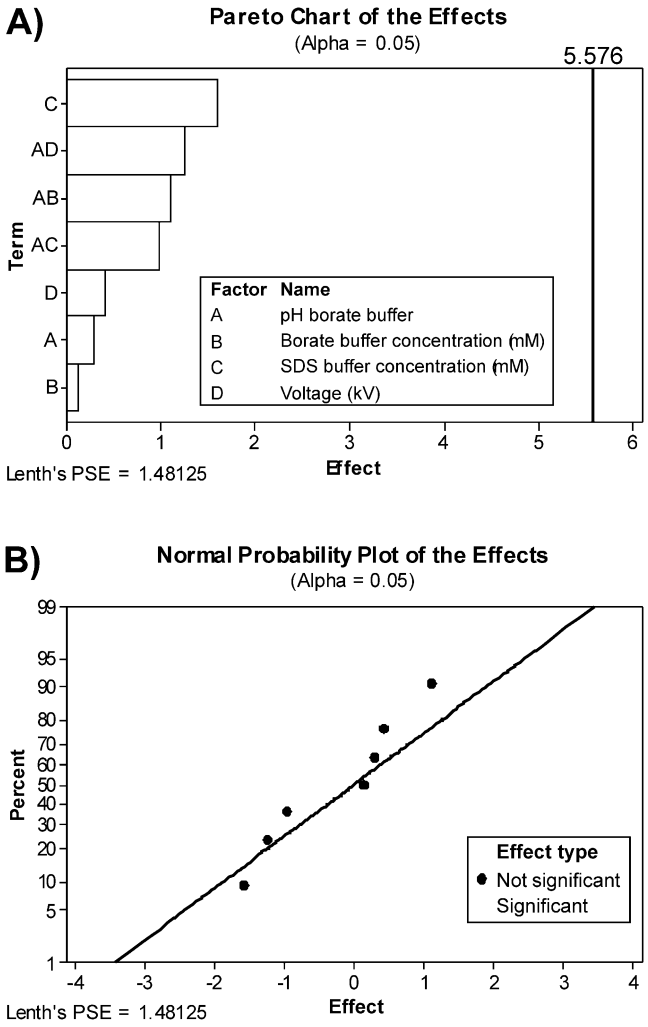

Figure 4. Pareto charts representing the effects of the variables and their interactions on the dronedarone assay for the robustness test using the fractional factorial design 2-Level 2^{4-1} (eight experiments) (A) and the normal probability plot of residuals for DRO (B).

3.2.7. Solution stability

Solution stability was carried out in order to determine its suitability for overnight analyses using autosamplers. The stability of the DRO sample solution was determined by placing the solution into the autosampler instrument for 12 h at room temperature. After this period, the stored solution was analyzed and compared with a freshly prepared reference solution. The assay results were less than 2% change relative to the content after the storage period.

3.2.8. System suitability

System suitability test was performed before validation experiments and during the routine analysis. Six reference solutions were analyzed in order to verify the repeatability of the system. Then, the parameters were measured and the following results were obtained: 25565 theoretical plates, resolution of 21.93, tailing factor of 1.03, migration time at 6.0 min and capacity factor (k') of 2.64. The parameter results indicated that the system performance is adequate for the analysis intended.

3.3. Application of the MEKC method to pharmaceutical analysis

The validated MEKC method was applied for the quantitative determination of DRO in commercially film-coated tablets. The analyses of five tablets were performed as described in Section 2.5. Assay results of the drug product were found to be 100.55% ± 0.39% (n = 5). The values obtained by the proposed method are in good agreement with the assay values reported by the manufacturer, which was ranged from 95.0 to 105.0% (AusPAR, 2010).

4. Conclusion

The proposed MEKC method was the first report of this kind of analytical separation technique for DRO. The validation results showed that the method is specific, linear, accurate, precise and robust, allowing DRO quantitation without any interference from degradation products or tablet excipients within a run time less than 10 min. The MEKC method was successfully applied for the quality control of DRO in commercially film-coated tablets, with

the advantages of reducing consumption of samples and organic solvents, thus reducing operating costs.

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ARTIGO 3 – DESENVOLVIMENTO E VALIDAÇÃO DE MÉTODO ESPECTROFOTOMÉTRICO PARA TESTE DE DISSOLUÇÃO DE DRONEDARONA EM COMPRIMIDOS

6 ARTIGO 3 – DESENVOLVIMENTO E VALIDAÇÃO DE MÉTODO ESPECTROFOTOMÉTRICO PARA TESTE DE DISSOLUÇÃO DE DRONEDARONA EM COMPRIMIDOS

Publicação científica: Development and validation of a spectrophotometric method for the dissolution test of dronedarone tablets.

Development and Validation of a Spectrophotometric Method for the Dissolution Test of Dronedarone Tablets

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Abstract

Dronedarone is a new antiarrhythmic agent amiodarone analogue, developed with the aim of reducing the side effects produced by its parent compound. It has been approved for the maintenance of normal heart rhythm in patients with atrial fibrillation and thereby reducing the risk of hospitalization. A dissolution test for dronedarone in film-coated tablets was developed with the following conditions: 900 mL of sodium acetate buffer pH 4.5, using USP Apparatus 2 (paddles) at a stirring rate of 75 rpm. The assay was performed by a UV-spectrophotometric method at 289 nm, which was validated and showed to be specific, linear ($r = 0.999$), precise (RSD < 2%) and accurate (mean recovery = 99.92%). Ahead of the results it can be concluded that the developed methods were efficient for dissolution test for dronedarone in film-coated tablets.

Keywords: Acetate buffer pH 4.5; Antiarrhythmic agent; Dissolution Test; Dronedarone; Paddles; Tablets; UV-Spectrophotometry; Validation.

INTRODUCTION

Atrial fibrillation (AF) is a cardiac arrhythmia that affects millions of people and is more commonly found in elderly patients (NATTEL, 2002; YALTA et al., 2009). The importance of this disease is related to the loss of atrial contractility, accelerated and inappropriate ventricular response and a deficient atrial appendage emptying favoring atrial stasis. Thus it leads to a significant increase in the predisposition of the risk of thrombus formation and, therefore, of thrombolysis (FAREH, 1998).

The conventional antiarrhythmic drugs for the treatment of atrial fibrillation have limited efficacy and safety, in addition of significant side effects and extra heart rate, make treatment in some case disadvantageous (NATTEL, 2002; CORLEY et al., 2004; STEINBERG et al., 2004; ZIMETBAUM, 2005; YALTA et al., 2009). In this context, substantial resources have been invested in developing of new antiarrhythmic drugs that reduce the toxicity and thereby improving the treatment of patients with atrial fibrillation (SINGH et al., 2010).

Dronedarone (Figure 1), a noniodinated benzofuran derivative, was developed for the treatment of patients with atrial fibrillation, reducing the rate of cardiovascular hospitalizations (SUN et al., 2002; HOHNLOSER et al., 2009). Dronedarone is an amiodarone analogue and differs from its parent compound by a removal of iodine and the addition of a methane-sulfonyl group. These changes reduced lipophilicity of the drug, thereby contributing to its shorter half-life (approximately 24 hours) and, therefore, its accumulation in tissue (ZAREBA, 2006). The molecular alterations referred to were made in order to minimize the risk of thyroid and pulmonary toxicity associated with amiodarone (WEGENER et al., 2006).

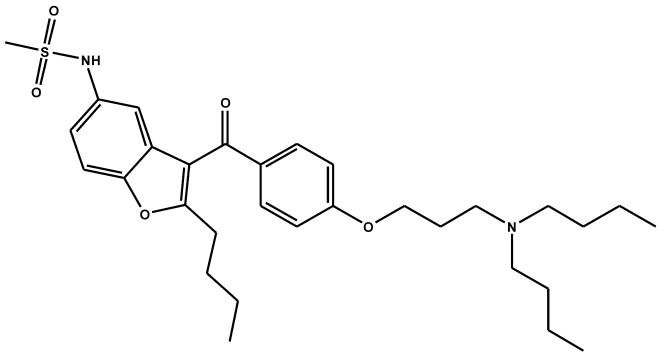

Figure 1 – Chemical structure of dronedarone.

The dissolution test is a tool used to evaluate the performance of a drug in solid form, with regard to their release from the dosage form. Dissolution is a physical test of a destructive nature, in which the drug passes to the soluble form from the intact dosage form (ABDOU, 1989; BANAKAR, 1992).

There are no dissolution test or UV-spectrophotometric method for dronedarone reported in the literature or listed in any pharmacopoeia, hence the importance of this work. The present study aims to develop and validate a UV-spectrophotometric method, which was applied to evaluate the proposed dissolution test and to determine the release profile of dronedarone in pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and instrumentation

Dronedarone hydrochloride reference standard (99.7%) was obtained from Sequoia Research Products Ltd. (Pangbourne, Berkshire, UK). Multaq® film-coated tablets (Sanofi-Aventis, France) were purchased from the market. Dronedarone hydrochloride film-coated tablets were claimed to contain 400 mg of the drug and the following excipients: hypromellose, cornstarch, crospovidone, poloxamer 407, lactose monohydrate, anhydrous colloidal silica, magnesium stearate, macrogol 6000, titanium dioxide and carnauba wax. All of the excipients were obtained from different local distributors.

Sodium acetate and hydrochloride acid (HCl) were obtained from Merck KGaA (Darmstadt, Germany). Polysorbate 80 and polysorbate 20 were purchased from Vetec (Duque de Caxias, Brazil). Monobasic potassium phosphate and sodium lauryl sulphate were purchased from Synth® (São Paulo, Brazil). LC-grade methanol was obtained from Tedia Company Inc (Fairfield, OH, USA). Water was purified using WaterPro™PS, Labconco system (Missouri, USA). Sodium acetate buffer (pH 4.5), monobasic potassium phosphate (pH 6.8) and hydrochloric acid were prepared according to the directions in United States Pharmacopeia (USP 34, 2011).

The dissolution test was carried out in a PharmaTest® multi-bath (n=6) dissolution system (Hamburg, Germany), in accordance with the United States Pharmacopeia under the general chapters (USP 34). A UV-VIS spectrophotometer model UV-1800 (Pró-Análise,

Porto Alegre, RS, Brazil), using 1.0 cm quartz cells, was used for the absorbance measurements.

Dissolution tests conditions

Solubility determination and sink conditions

Solubility is a relevant parameter in selection of the dissolution medium. Drug solubility was determined in different medium and expressed, in mg/mL, the amount of dissolved drug (ABDOU, 1989; BROWN, 2004). To establish *sink conditions*, the solubility of dronedarone was determined in different media: 0.1 M HCl, sodium lauryl sulfate (0.1, 0.5, 1.0 and 2.0%, w/v), potassium phosphate buffer (pH 4.0, 4.5 and 6.8), polysorbate 20 (2.0%, w/v), polysorbate 80 (0.1, 0.5, 1.0, 2.0%, w/v) and sodium acetate buffer (pH 4.5). An excess of dronedarone (20 mg) was transferred to vessels containing 10 mL of dissolution medium (maintained at 37 °C) under constant stirring, until precipitation of the drug occurred. Undissolved drug was removed by centrifugation. An aliquot (1 mL) of the resulting solution was diluted with medium at a final concentration of 10 µg/mL and then, analyzed by spectrophotometer.

In vitro drug release study

The release characteristics of the film-coated tablets were determined using USP Apparatus II (paddle) at 75 rpm in 900 ml of medium maintained at 37 ± 0.5°C. The dissolution media consisted of sodium acetate buffer (pH = 4.5) (Dissolution test I) and 1.0% (w/v) polysorbate 80 in water (Dissolution test II). Each dissolution test was performed with 12 film-coated tablets, using manual sampling aliquots of 5 mL which were withdrawn at time intervals of 15, 30, 45, 60, 75 and 90 min. To maintain a constant total volume, fresh medium at 37°C was replaced in the same volume. In dissolution test I, the samples were centrifugated at 4000 rpm during 10 minutes; otherwise, in dissolution test II they were filtered. Aliquot of 1mL was transferred to 25 mL volumetric flask and diluted with each medium obtaining the final concentration 17.77 µg/mL. The samples solutions were analyzed by UV-spectrophotometry to characterize the dissolution profile of dronedarone.

Analysis of dissolution profiles

The drug releases profiles of Dissolution Test I (sodium acetate buffer pH 4.5) and Dissolution Test II (1.0% polysorbate 80) were compared by a model independent approach using the difference factor ($f1$) and the similarity factor ($f2$). Twelve samples were assayed for each dissolution media (maintained at 37 ± 0.5°C) using USP Apparatus II (paddle) at a rate of 75 rpm. Five milliliters of the dissolution medium were withdrawn at 15, 30, 45, 60, 75 and 90 min and after analyzed by the spectrophotometric method. For the curves to be considered similar the $f1$ factor values must be within 0-15, which measures the percentage error between curves over all time points. The similarity factor ($f2$) is a logarithmic transformation of the sum-squared error of differences between the profiles over all time points, and $f2$ values within 50-100 ensure equivalence of the two curves (MOORE; FLANNER, 1996; FDA, 1997). The dissolution profiles were also compared by calculation of DE, which was defined as the area under the dissolution curve at time t, measured using the trapezoidal rule, and expressed as percentage of the area of the release rectangle described by 100% dissolution in the same time (KHAN, 1975). The results were submitted to analysis of variance (one-way ANOVA). Differences between means were considered to be statically significant at a $p < 0.05$.

Preparation of reference solution

The reference stock solution of dronedarone was prepared by accurately weighing 10 mg of dronedarone hydrochloride transferred to a 50 mL volumetric flask and diluted with methanol (200 µg/mL). This stock solution was stored at 2-8°C and daily diluted with dissolution medium to obtain a final concentration of 15 µg/mL.

Preparation of sample solution

Twenty Multaq® tablets were weighed and thoroughly crushed. An amount of tablet powder equivalent to 15 mg of dronedarone was transferred to a 50 mL volumetric flask and diluted with sodium acetate buffer (pH 4.5). This solution was sonicated for 15 min. Aliquot of 1 mL was transferred to a 20 mL volumetric flask and diluted with sodium acetate buffer (pH 4.5) to obtain a final concentration of 15 µg/mL.

UV validation

In order to ensure that the proposed spectrophotometric method is appropriate to dissolution test, it was validated through the analysis of specificity, linearity, precision, accuracy and robustness parameters according to USP Pharmacopeia (USP 34, 2011) and ICH guidelines (ICH, 2005).

Specificity

To ensure that the spectrophotometric method is specific, a placebo (in-house mixture of all excipients) was prepared. Specificity was determined by the dissolution of placebo in 900 mL of sodium acetate buffer (pH 4.5) for 90 minutes. The UV-visible absorption spectra of placebo and sample after 90 min of dissolution test were obtained, by the proposed method, in order to verify the interference from formulation components or dissolution medium in the determination of dronedarone.

Linearity

To demonstrate linearity of the method, three independent analytical curves were constructed plotting concentration (µg/mL) *versus* absorption. Appropriate amounts of the reference solution (50 µg/mL) were diluted with sodium acetate buffer (pH 4.5) to give concentrations in the range from 2.5, 5.0, 15.0, 20.0 to 25.0 µg/mL. The absorbance values were plotted against the respective concentrations of dronedarone to obtain the analytical curves. The linearity was evaluated by linear regression analysis, which was calculated by the method of least squares, and by analysis of variance (ANOVA).

Precision

The precision of the method was evaluated by repeatability (intra-assay) and intermediate precision (inter-day precision), expressed as relative standard deviation (RSD). Repeatability was assessed using six determinations at the same concentration of dronedarone (15 µg/mL), under the same experimental conditions (same day and analyst). Intermediate precision is studied by the analysis performed with within-laboratories variations such as

different days, analysts and equipments (ICH, 2005). Intermediate precision was determined by comparing the results from six determinations at the same concentration (15 µg/mL), performed in two different days and between-analysts.

Accuracy

The accuracy of the spectrophotometric method was expressed as percent recovery by the assay of known added amounts of reference solution in the sample (ICH, 2005). An amount of tablet power equivalent of 10 mg of dronedarone was transferred to a 100 mL volumetric flask and dissolved with sodium acetate buffer (pH 4.5) (final concentration of 100 µg/mL). Aliquot of 1 mL of this solution was transferred to 20 mL volumetric flasks, which contained 1.0, 2.0 and 3.0 mL of dronedarone reference solution (100 µg/mL). The samples were diluted with sodium acetate buffer (pH 4.5) to obtain final concentrations of 10.0, 15.0 and 20.0 µg/mL, which correspond to 66, 100 and 133% of the test concentration.

Robustness

The robustness of the spectrophotometric method was evaluated by analysis of samples under different analytical conditions such as changing the wavelength (287-291 nm) and the sodium acetate buffer pH value (4.3-4.7).

Drug stability

The stability of solutions in dissolution medium was analyzed by leaving them into the dissolution vessels from 24h, protected from the light. The samples were prepared at the same conditions of the dissolution test. The content of dronedarone in solution was determined by UV-spectrophotometry and percentages between 98 and 102% of the initial value indicated the drug stability.

RESULTS AND DISCUSSION

Solubility determination

Dronedarone aqueous solubility is limited: 0.64 mg/mL at ambient temperature (AusPAR, 2010). Figure 2 shows the solubility results of dronedarone at different pH levels and surfactants. The solubility tests showed precipitation of dronedarone in 0.1 M HCl and potassium phosphate buffer (pH 6.8) and then this media were discarded. In the presence of a sodium acetate buffer (pH 4.5) solubility increases to 2.02 mg/mL. Dronedarone solubility in sodium acetate and potassium phosphate buffers increases in pH range from 4.0 to 5.0 and it decreases to virtually zero in pH 6.8 potassium phosphate buffer. The drug has a higher solubility in polysorbate 80 at 1.0 and 2.0% (1.88 mg/mL and 2.10 mg/ml, respectively).

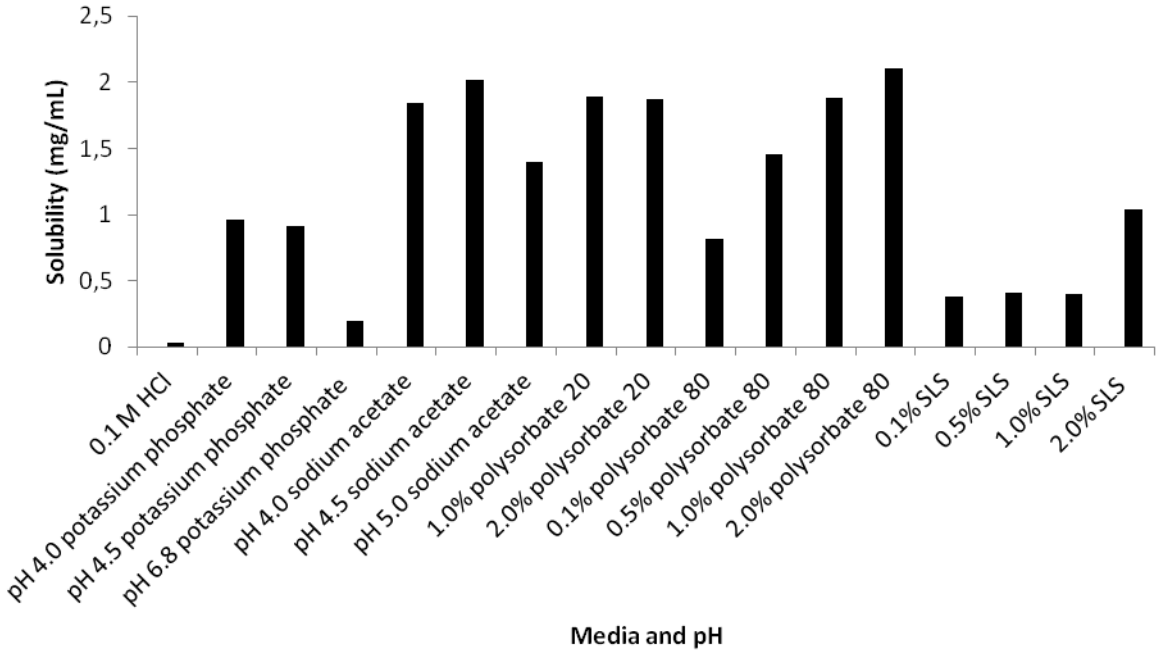

Figure 2 – Solubility of dronedarone hydrochloride in different media.

Dissolution test design

The dissolution test is the most important method to study, under *in vitro* conditions, the transfer of drug from a solid dosage form, thus representing an important tool to evaluate the factors that influencing the bioavailability of a drug. During the dissolution test, the cumulative amount of drug which passes into solution is recorded as a function of time. Thus, the test describes the overall speed of all the processes involved in release of drug from a bioavailable form (AULTON, 2005).

The dissolution tests aim to ensure quality from batch to batch, guide the development of new formulations, ensure uniformity of quality and performance of the drug after certain changes and indicate potential problems of bioavailability (FDA, 1997).The aim of the dissolution test is to find an *in vitro* characteristic of a potential formulation that reflects their performance *in vivo*. The establishment of correlations between *in vitro*/ *in vivo* may be advantageous from the standpoint of ethical, economic and technical help and can replace or reduce the bioavailability studies in humans (COSTA; LOBO, 2001). For this purpose, the design of a dissolution test needs to reproduce the physiological conditions in the gastrointestinal tract after the administration of the dosage form (DRESSMAN et al., 1998).

During dissolution testing design, some important parameters were taken into account to guide the choice of a dissolution medium. Drug solubility is among these parameters. Considering dronedarone solubility results mentioned above, the tablets were tested in 900 mL of pH 4.0 and pH 4.5 sodium acetate buffers, pH 4.0 and pH 4.5 potassium phosphate buffers, 1.0 and 2.0 % polysorbate 80 (Figure 3). As the paddle method (USP Apparatus 2) is normally used for the dissolution test of solid oral dosage forms, according to USP 34 (2011), the test conditions were selected based on a screening study using this dissolution test apparatus.

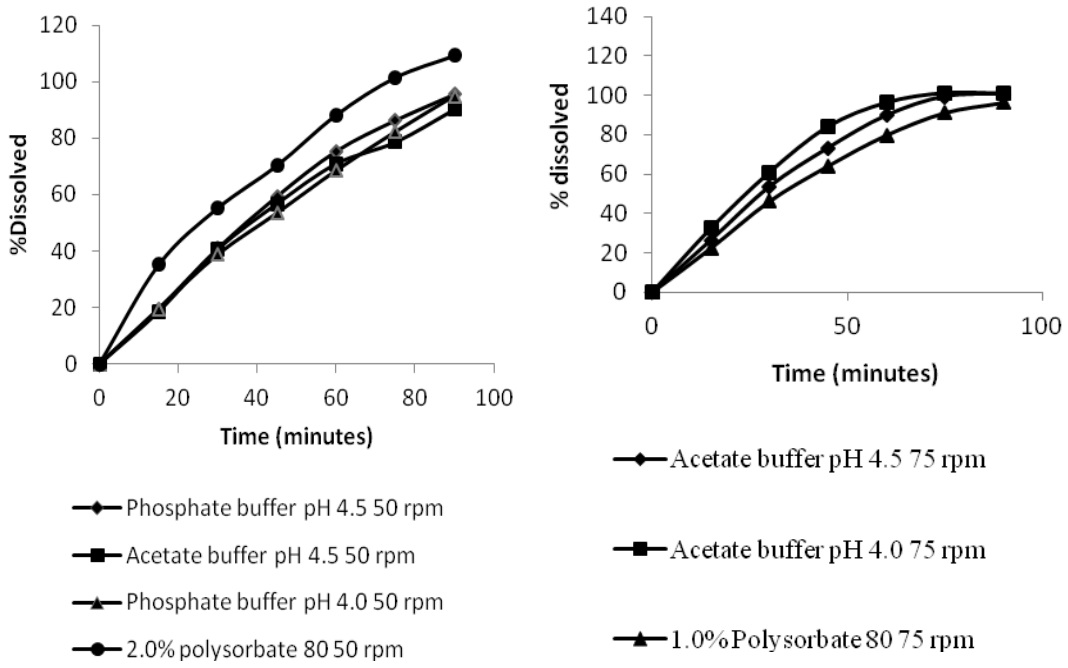

Figure 3. Dissolution profiles of dronedarone tablets using sodium acetate buffer pH 4.5, phosphate buffer pH 4.0 and 4.5 and 2.0 % polysorbate 80 in USP Apparatus 2 rotating at 50 rpm and dissolution profiles of dronedarone tablets using pH 4.5 and 4.0 sodium acetate buffers and 1.0% polysorbate 80 in USP Apparatus 2 rotating at 75 rpm.

Besides the dissolution profile of dronedarone in sodium acetate buffer pH 4.5 using USP Apparatus 2 at a stirring rate of 50 rpm, the drug was tested in the same condition by changing the stirring rate to 75 rpm to obtain better results. From the Figure 3, it can be seen that the dissolution rate increased as the USP Apparatus 2 rotation speed also increased. Due to the fast drug release, the agitation speed of 75 rpm was chosen for USP Apparatus 2.

Considering that dronedarone solubility was higher in pH 4.5 sodium acetate buffer and 1.0% polysorbate 80, these two media were used for the dissolution test, which were referred as Dissolution test I and II, respectively. The dissolution medium containing 2.0% polysorbate 80 was discarded due to low reproducibility at 75 rpm.

Analysis of dissolution profiles

The similarity between the dissolution profiles was assessed by a simple independent model approach using the difference factor ($f1$) and the similarity factor ($f2$), which were adopted by the FDA Center for Drug Evaluation and Research (FDA, 1997) and the Human Medicines Evaluation Unit of The European Agency for the Evaluation of Medicinal Products

(EMEA) (COSTA; LOBO, 2001). For the curves to be considered similar the $f1$ factor values must be within 0-15, which measures the percentage error between curves over all time points. The similarity factor ($f2$) is a logarithmic transformation of the sum-squared error of differences between the profiles over all time points, and $f2$ values within 50-100 ensure equivalence of the two curves (MOORE; FLANNER, 1996; FDA, 1997).

The dissolution profiles of Dissolution test I (sodium acetate buffer pH 4.5) and Dissolution test II (1.0% polysorbate 80) using USP Apparatus 2 were compared under the same conditions: same dissolution time points including only one measurement after 85% dissolution and relative standard deviations less than 20% at 15 min and less than 10% at other time points. Despite the similarity test resulted in 10.53 for $f1$ (difference factor) and 56.47 for $f2$ (similarity factor), concluding that the two dissolution profiles were considered similar, the opposite was verified through dissolution efficiency (DE). The DE was calculated for the two dissolution profiles in 60, 75 and 90 min time points (Table 1). One-way ANOVA of the DE% values showed that the percent of drug released was not similar between the two media and the sodium acetate buffer pH 4.5 was the one with the higher DE%.

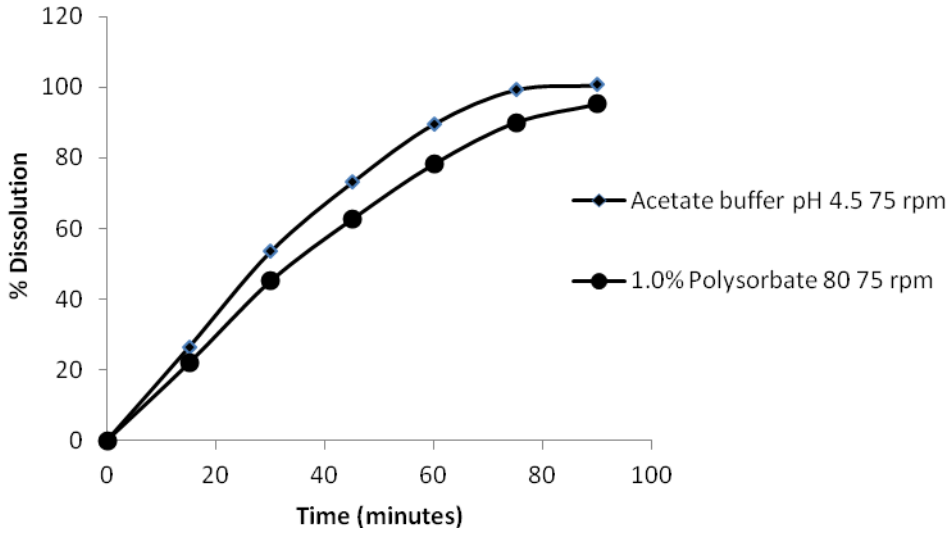

Figure 4. Dissolution profiles of dronedarone tablets using sodium acetate buffer pH 4.5 and 1.0% polysorbate 80 in apparatus 2 rotating at 75 rpm (n = 12).

Table 1 – Comparison of dissolution profiles of dronedarone in different media through the dissolution efficiency (DE%).

Time (min)	Acetate pH 4.5 DE% ± RSD	1.0% Polysorbate 80 DE% ± RSD
60	33.42 ± 6.5	28.72 ± 4.8
75	49.17 ± 5.1	42.96 ± 3.9
90	65.83 ± 3.9	58.59 ± 3.2

Media selection

Dronedarone is a compound with low solubility. For poorly water-soluble drugs, the main limitation to oral absorption is the dissolution rate, because there is not enough GI fluids to dissolve the dose administrated (DRESSMAN et al., 1998; DRESSMAN; REPPAS, 2000). Dronedarone is recommended to be administrated twice a day with food because it increases the absorption and therefore its bioavailability. However, dronedarone is poorly absorbed in the fasted state (HOY; KEAM, 2009).

Gastric pH values after meal ingestion are in the range 3.0 to 7.0. In the small intestine in the fed state, the pH value is around to pH 5 in the duodenum. The medium used to represent fed state in the proximal GI tract contains an acetate buffer instead of a phosphate buffer, due to the fact that the simulation of the conditions in the upper small intestine must be made with the higher buffer capacity maintaining the low pH value. The volume of up to 1L represents the meal-induced secretions. In the fed state, gastric emptying times for nondigestible solids vary according to the size of the solid particles and the meal (DRESSMAN et al., 1998).

According to the literature for immediate release solid oral dosage forms, a two-point dissolution specification (one at 15 min and other at 60 min) is indicated to assess 85% of drug dissolved for poorly water soluble drugs (FDA, 1997). As shown in Fig. 4, 89% of dronedarone dissolved at 60 min for Dissolution test I (pH 4.5 sodium acetate buffer). However, only 79% of dronedarone dissolved at 60 min for Dissolution test II (1.0% polysorbate 80).

Based on the parameters presented previously, the Dissolution test I was selected because the drug released profile obtained was considered most satisfactory, with higher DE% and more than 85% drug has dissolved at 60 min. The conditions for the Dissolution test I for

dronedarone in tablets were: 900 mL of sodium acetate buffer pH 4.5 using the paddle method at stirring rate of 75 rpm.

Validation of the method of quantification

UV/VIS spectrophotometry is the traditional analytical method for quantifying drug release in dissolution tests. Furthermore, it is a simple and fast analysis and smaller amounts of solvents are used, which makes it a low-cost method for the routine quality control (WANG; HIGGINS, 2006; VAUCHER et al., 2009). The spectrophotometric method was selected to determine the dissolution profile due to the fact that there is no interference from the excipients in the absorption maximum of dronedarone.

Specificity

Specificity of the method was demonstrated by comparing the UV-visible absorption spectra of reference solution, placebo and sample after 90 min of dissolution test. From the absorption spectra (Figure 5), it was found that there was no interference from excipients or dissolution medium in the absorption maximum of dronedarone at 289 nm, demonstrating that the proposed method is specific. Thus, it allows the rapid and accurate determination of dronedarone in film-coated tablets.

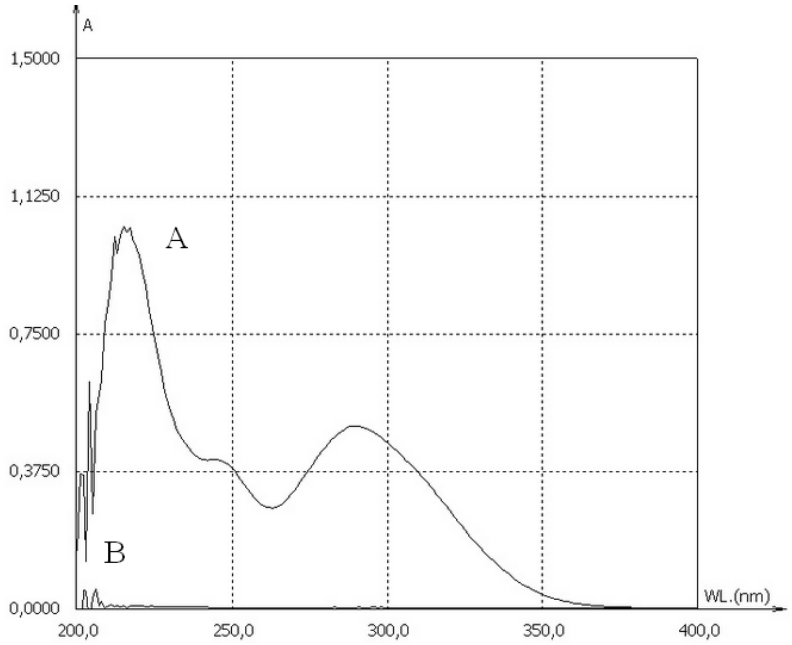

Figure 5 - UV spectrum of dronedarone sample (A) and placebo solutions (B) after 90 minutes of dissolution test.

Linearity

The UV-spectrophotometric method was considered to be linear in the range from 2.5 to 25.0 µg/mL, which corresponds from 16.7 to 166.7% of the test concentration of 15 µg /mL. The correlation coefficient has been obtained (r = 0.9998), with the linear equation: y = 0.0315x – 0.0117. The calibration data were validate by means of ANOVA that demonstrated no significant linearity deviation (f calculated < f critical) (p < 0.05).

Precision

The results of the repeatability (intra-assay) and intermediate precision (inter-day precision) are shown in Table 1. The method repeatability resulted in a mean value of 99.60% and a relative standard deviation (RSD) of 0.21% (n = 6). Intermediate precision was assessed by analyzing six sample solutions in two different days and the RSD value obtained was 0.25% (n = 12). Between-analyst precision was determined by calculating the RSD for the analysis of six sample solutions by two analysts on the same day; the RSD was found to be 0.31% (n = 12). The results are in agreement with the published in the literature showing a good precision of the spectrophotometric method for dissolution test.

Table 2. Intra-assay and intermediate precision for the dronedarone samples in pharmaceutical dosage form using the UV spectrophotometric method.

Intra-assay precision Experimental amount (%) ± RSD	Intermediate precision Experimental amount (%) ± RSD	
1st Day – Analyst I	2nd Day – Analyst I	2nd Day – Analyst II
99.58 ± 0.29	99.79 ± 0.21	99.38 ± 0.21
99.38 ± 0.31	100.21 ± 0.35	99.93 ± 0.12
99.59 ± 0.20	99.38 ± 0.36	100.35 ± 0.24
99.38 ± 0.75	99.52 ± 0.12	99.65 ± 0.52
99.79 ± 0.31	99.52 ± 0.78	99.59 ± 0.29
99.89 ± 1.03	99.72 ± 0.12	99.65 ± 0.12

Accuracy

The accuracy was calculated as the percentage of recovery of known added dronedarone reference solution. The recoveries obtained ranged from 98.0 to 101.8% (99.9 ± 1.13%, mean ± RSD%, $n = 9$) for the film-coated tablets, demonstrating that the accuracy was considered acceptable. Results of accuracy of the spectrophotometric method were shown in Table 3.

Table 3. Accuracy results for the spectrophotometric method applied to dronedarone pharmaceutical dosage form.

Added (µg/mL)	Recovered* (µg/mL)	Recovery* (%)	RSD (%)
5.0	4.99	99.9	0.79
10.0	9.93	99.3	1.25
15.0	15.07	100.5	1.40

* each value is the mean of 3 determinations

Robustness

The robustness of the UV-spectrophotometric method was studied by the analysis of samples under small variations in method conditions. The results are given in Table 4. Deliberate variations in analytical parameters, such as wavelength and sodium acetate buffer pH value, did not lead to a significant effect on determination of dronedarone in pharmaceutical dosage form, indicating the robustness of the proposed method.

Table 4. Conditions and range investigated during robustness testing.

Variable	Range investigated	Dronedarone%	Optimal conditions
Wavelength	287 nm	98.14	
	289 nm	99.59	289 nm
	291 nm	98.56	
Buffer pH	4.3	100.30	
	4.5	99.80	4.5
	4.7	100.10	

Drug stability

Dronedarone was found to be stable in dissolution medium for 24h when maintained at room temperature and protected from the light. The results demonstrated that the sample solution remained at 100.85 ± 1.4% over a period of 24h.

CONCLUSION

The dissolution test developed for dronedarone tablets was considered satisfactory. The best conditions that allowed the dissolution determination were obtained using 900 mL of pH 4.5 sodium acetate buffer (maintained at 37.0 ± 0.5 ºC) with USP Apparatus 2 (paddle) at a stirring speed of 75 rpm. The Dissolution test I (pH 4.5 sodium acetate buffer) showed to be

reliable due to the higher dissolution efficiency (DE%) and adequate release of dronedarone from the drug product, according to dissolution specifications. The spectrophotometric method was validated and showed to be specific, linear, precise and accurate. The dissolution test and the UV-spectrophotometric method demonstrated to be adequate to evaluate the release profile and could be used in quality control of dronedarone film-coated tablets, collaborating to the official codes.

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DISCUSSÃO

7 DISCUSSÃO

A necessidade de se mostrar a qualidade de medições analíticas, através de sua comparabilidade, rastreabilidade e confiabilidade, está sendo cada vez mais reconhecida e exigida. Dados analíticos não confiáveis podem conduzir a decisões desastrosas e a prejuízos financeiros irreparáveis (RIBANI et al., 2004). Nesse contexto, torna-se necessário validar o método analítico, a fim de garantir que este é adequado para a análise pretendida, assegurando a confiabilidade dos resultados (BRASIL, 2003; ICH, 2005).

Em 2009, a dronedarona foi aprovada pelo FDA e EMA e, então foi lançada no mercado americano e europeu e não há publicações sobre métodos para a sua análise quantitativa em forma farmacêutica ou matéria-prima.

7.1 Método por cromatografia líquida

A cromatografia líquida é geralmente o método de escolha para o controle de qualidade de formas farmacêuticas. Além de permitir a separação de múltiplos componentes, os métodos cromatográficos possuem a vantagem de possuir uma maior exatidão e sensibilidade mesmo para pequenas quantidades de produtos de degradação produzidos (BAKSHI; SINGH, 2002).

O método por cromatografia líquida em fase reversa (CL-FR) foi desenvolvido e validado para determinação de dronedarona em forma farmacêutica e, para esse fim, alguns parâmetros foram estudados tais como: diferentes marcas de coluna C18, seleção do solvente, composição e pH da fase móvel. conforme descrito no **ARTIGO 1**. O método foi otimizado para se obter um método indicativo de estabilidade.

Optou-se pelo emprego de uma coluna C18 devido a solubilidade da dronedarona em solventes orgânicos, que possibilitou o emprego de fase reversa. Foram testadas diferentes fases móveis utilizando solução tampão pH 4,9/metanol/acetonitrila em diferentes proporções. A fase móvel composta por solução tampão pH 4,9: acetonitrila (35:65, v/v) foi a de escolha, pois nessas condições foram obtidas boa eficiência (> 9000 pratos teóricos) e simetria (1,21), além de um tempo de retenção reprodutível de 5,2 minutos, permitindo uma determinação rápida de dronedarona, importante na rotina do controle de qualidade. A especificidade do

método foi verificada através do preparo de um placebo (contendo todos os excipientes da forma farmacêutica) e através dos estudos de degradação forçada, onde uma solução da amostra de dronedarona foi submetida a condições de estresse como: hidrólise ácida, hidrólise básica, oxidação, fotólise e calor. Os cromatogramas, obtidos nos estudos de degradação forçada (Figura 2) e do placebo (Figura 4), demonstram que não há picos interferindo na determinação de dronedarona, o que foi confirmado através do uso de testes de pureza de pico (obtido com o detector de arranjo de fotodiodos), nos quais foram obtidos valores de pureza de pico superiores a 0.9998. O método cromatográfico foi linear no intervalo de concentrações de 5 a 100 µg/mL, que corresponde a 25 – 500% da concentração do teste. A regressão linear foi obtida através do método dos mínimos quadrados, com a equação da reta y = 44111,42x + 21073,45 e o coeficiente de correlação de 0,9999. Os resultados obtidos nas três curvas analíticas foram submetidos à análise de variância, demonstrando que há regressão linear significativa e que não houve desvio da linearidade. A precisão foi determinada em termos de repetibilidade e precisão intermediária. Os desvios padrão relativo obtidos na repetibilidade e precisão intermediária foram 0,69% (n = 6) e 0,61% (n = 18), respectivamente, demonstrando a precisão do método cromatográfico (Tabela 1). A exatidão do método foi verificada pelo método de adição de padrão e o valor da média de recuperação foi de 99,30% (DPR = 0,59; n = 9). Os valores obtidos no teste de recuperação encontram-se dentro do intervalo preconizado na literatura de 98 a 102% (Tabela 2). (SHABIR, 2003). Os valores dos limites de detecção e quantificação foram obtidos através da equação foram 0,32 e 0,96 µg/mL; experimentalmente os valores de LOD e LOQ foram 1,0 e 5,0 µg/mL. A robustez do método foi avaliada através da realização de pequenas variações nas condições cromatográficas como na proporção de acetonitrila, pH e fluxo da fase móvel (Tabela 3). Não foram observadas diferenças significativas nos teores de dronedarona das amostras analisada nas diferentes condições, demonstrando, assim, que o método é robusto. No teste de adequabilidade do sistema, foram obtidos os seguintes resultados: número de pratos teóricos (N) igual a 9154; fator de cauda (T) de 1,21; fator de capacidade (k') de 2,34 e DPR entre as áreas do pico igual a 0,45%. Esses resultados se encontram dentro dos valores preconizados ($N > 2000$; $T \leq 2$; $k' > 2$; DPR $\leq 1\%$) indicando que o sistema é adequado para a análise pretendida (SHABIR, 2003; USP, 2011). A partir dos resultados obtidos, conclui-se que o método cromatográfico proposto cumpre os requisitos descritos na literatura e, portanto, é adequado para a determinação quantitativa de dronedarona em comprimidos revestidos.

7.2 Método espectrofotométrico no UV

O método espectrofotométrico utilizando metanol como diluente foi desenvolvido como uma alternativa rápida, de baixo custo e com instrumentação mais simples em relação à CL-FR, para a determinação quantitativa de dronedarona em comprimidos revestidos, conforme demonstrado no **ARTIGO 1**. Durante o desenvolvimento do método, empregou-se o metanol como diluente, considerando a boa solubilidade da dronedarona nesse solvente. O método foi validado de acordo com o ICH (2005). A especificidade do método espectrofotométrico foi verificada através da comparação entre os espectros obtidos a partir da solução referência e da solução amostra contendo dronedarona com o espectro do placebo (mistura dos excipientes do comprimido). De acordo com os espectros obtidos (Figura 5), não houve interferência dos excipientes em um dos máximos de absorção da dronedarona, 289 nm, o comprimento de onda escolhido. A linearidade foi determinada através da construção de três curvas analíticas no intervalo de 5,0 a 25,0 µg/mL. A equação da reta obtida foi y = 0,0326x + 0,0144 com coeficiente de correlação de 0,9999. A precisão foi avaliada através da repetibilidade, expressa como o DPR de 0,77% (n = 6) e precisão intermediária, com valores de DPR entre dias e entre analistas de 0,59% (n = 18) e 0,66% (n = 12), respectivamente (Tabela 4). A exatidão foi determinada pelo método de adição de padrão e os valores de recuperação obtidos se encontram dentro do intervalo de 98-102% (Tabela 5). Os valores de limite de detecção e limite de quantificação foram calculados a partir do desvio padrão do intercepto com o eixo Y e pela inclinação da curva analítica; os resultados obtidos foram 0,44 e 1,32 µg/mL, respectivamente. A robustez foi avaliada pela análise das soluções referência e amostra utilizando diferentes comprimentos de onda e marcas de solvente; não houve diferenças significativas nos teores obtidos, demonstrando que o método é robusto. Dessa forma, o método espectrofotométrico pode ser aplicado na determinação de dronedarona em forma farmacêutica. Os valores experimentais obtidos pelo método espectrofotométrico foram comparados estatisticamente pelo teste “t” de Student com os valores do método por CL-FR (Tabela 6). Não houve diferença significativa entre os valores experimentais obtidos na análise de doze soluções de amostra de comprimidos contendo dronedarona pelos dois métodos. Portanto, conclui-se que ambos são adequados para a determinação quantitativa de dronedarona em comprimidos revestidos.

7.3 Método por cromatografia eletrocinética micelar

Os métodos cromatográficos são geralmente empregados na análise de espécies neutras insolúveis em água, já que a eletroforese capilar de zona não pode ser aplicada para separar compostos neutros, pois estes migram em uma mesma banda ou zona nessa técnica. O reconhecimento dessa limitação levou ao desenvolvimento da cromatografia eletrocinética micelar. A eletrocromatografia capilar tem se mostrado capaz de gerar separações altamente eficientes e rápidas de compostos neutros insolúveis (ALTRIA, 1999). A cromatografia eletrocinética micelar é um método simples baseado na solubilização micelar empregando o mesmo equipamento de EC. Utiliza um surfactante que contem tanto grupos hidrofóbicos como hidrofílicos, adicionado a solução eletrolítica em concentração acima da concentração micelar crítica para formar micelas, as quais atuam como uma fase pseudo-estacionária. O mecanismo de separação dos analitos é baseado na partição entre a micela e a solução eletrolítica; os compostos neutros inserem-se no interior das micelas e os compostos ionizados permanecem no meio condutor (TERABE, 2004).

O método por eletroforese capilar, utilizando modo de separação por cromatografia eletrocinética micelar, foi validado, empregando a nimesulida como padrão interno, conforme demonstrado no **ARTIGO 2**. O método foi otimizado através de modificações nos parâmetros: pH da solução eletrolítica, concentração do tampão borato, concentração de SDS e efeitos da temperatura, do tempo de injeção e da voltagem aplicada. Optou-se pelo uso de uma solução eletrolítica composta por tampão borato 40 mM e SDS 50 mM pH 9,2 com detecção em 216 nm. Empregou-se um capilar de sílica fundida de 40 cm de diâmetro efetivo e as amostras foram injetadas no modo hidrodinâmico a 50 mbar por 7 s com voltagem de 28 kV. Essa condição otimizada permitiu a obtenção de picos com boa simetria e resolução e com tempos de migração adequados, em uma corrida analítica de 7,0 minutos. A possível interferência a partir dos excipientes do comprimido e dos produtos de degradação na quantificação de dronedarona foi estudada obtendo-se os eletroferogramas de amostras submetidas à degradação forçada (Figura 2) e do placebo (Figura 3). A partir desses eletroferogramas, observa-se que nenhum pico adicional migrou com o pico da dronedarona, o que foi confirmado através do teste de pureza de pico. A linearidade foi determinada no intervalo de concentrações de 25 a 150 µg/mL (r = 0,9995). O valor experimental médio obtido na exatidão foi de 99,97% (DPR = 1.09%; n = 9), indicando que o método é exato dentro do intervalo esperado (Tabela 2). Na avaliação da precisão do método, o DPR obtido

na repetibilidade foi de 0,67% e o valor de DPR entre dias foi de 0,65% (Tabela 3), ambos de acordo com a literatura que preconiza o valor de DPR igual ou inferior a 2% (SHABIR, 2003). Na determinação da robustez do método (Tabela 1), a significância dos efeitos dos fatores foi avaliada através do diagrama de Pareto, no qual se observou que não há desvio de cada fator e, portanto, que as alterações não foram significativas (Figura 4). O teste de adequabilidade do sistema foi executado a fim de avaliar a reprodutibilidade deste e os resultados obtidos foram satisfatórios, indicando que o mesmo é adequado para a análise pretendida. Portanto, conclui-se que o método por MEKC cumpre os requisitos exigidos na literatura e pode ser aplicado no controle de qualidade para determinação de dronedarona em comprimidos revestidos.

7.4 Comparação dos métodos validados por cromatografia líquida, espectrofotometria no UV e cromatografia eletrocinética micelar para a avaliação de dronedarona em comprimidos revestidos

Os métodos cromatográfico, espectrofotométrico e eletroforético (MEKC) foram aplicados na determinação do teor de dronadarona em comprimidos revestidos. Os valores experimentais obtidos pelos métodos propostos foram comparados estatisticamente por análise de variância (ANOVA) e os resultados demonstraram que não há diferença significativa entre os métodos propostos ($p > 0,05$), com teores médios 0,58% e 0,54% maiores para o método cromatográfico em relação aos métodos espectrofotométrico e eletroforético, respectivamente. Desse modo, observa-se que todos os métodos analíticos podem ser aplicados na análise quantitativa de comprimidos contendo dronedarona. A comparação dos métodos analíticos representa uma contribuição importante para se estabelecer novas alternativas na rotina do controle de qualidade de formas farmacêuticas.

7.5 Estudos de degradação forçada

O estudo de degradação forçada ou teste de estresse é definido como um teste de estabilidade para fármacos e medicamentos sob condições extremas, tais como condições de

temperatura, umidade e intensidade de luz que podem causar a decomposição destes. Essas condições extremas aumentam a deterioração do produto e por isso reduzem o tempo necessário para o teste, permitindo que mais dados sejam coletados, o que é usado na seleção da melhor formulação (AULTON, 2005). A realização do teste de degradação forçada é de grande importância para as indústrias farmacêuticas, pois este deve ser contemplado em conjunto com sua análise crítica, no momento do registro, pós-registro e renovação (SILVA et al., 2009). O teste de estresse pode ajudar na identificação de prováveis produtos de degradação, além de auxiliar a estabelecer as vias de degradação, a estabilidade intrínseca da molécula e a validar a capacidade indicadora de estabilidade dos métodos analíticos utilizados. O teste de estresse deve ser realizado em um único lote do medicamento e deve incluir o efeito da temperatura, umidade, oxidação e fotólise do fármaco, além de incluir a suscetibilidade da substância à hidrólise (ICH, 2003).

Os testes de degradação forçada foram realizados para o desenvolvimento e validação do método cromatográfico, a fim de demonstrar a capacidade do método de ser indicativo de estabilidade, considerando-se que a CL-FR é o método mais empregado em estudos de estabilidade devido a alta capacidade de resolução, sensibilidade e especificidade (BAKSHI; SINGH, 2002). Para realizar o estudo de degradação forçada em condições hidrolíticas, utilizou-se o ácido clorídrico (HCl) a 3,0 M a 80°C por 7 h e o hidróxido de sódio (NaOH) a 1,0 M a 80°C por 30 minutos. Na hidrólise ácida, houve degradação de aproximadamente 7,5% do fármaco, com formação de um produto de degradação que foi detectado em 4,11 minutos. Na hidrólise básica, houve formação de um pico adicional em 2,8 minutos. Na degradação oxidativa, a reação de solução de dronedarona com o peróxido de hidrogênio a 30% em temperatura ambiente por 48 h promoveu uma degradação de 22% do fármaco e a formação de um produto de degradação com tempo de retenção de 4,42 min. O estudo de fotoestabilidade foi realizado através da exposição da amostra de dronedarona a luz UVA por 24 h e UVC por 1h30 min, onde houve um decréscimo da área do pico de 26% e 23%, respectivamente, e não foi detectado nenhum pico adicional nessas condições. A dronedarona manteve-se estável na condição neutra térmica quando submetida a 80°C por 6 h.

A cinética de degradação alcalina da dronedarona foi estudada através da diluição de amostras de dronedarona em hidróxido de sódio a 1,0 M, que foram colocadas em banho termostatizado a 60°C, retiradas em intervalos pré-estabelecidos e neutralizadas antes da análise por CL-FR. A degradação alcalina da dronedarona segue cinética de primeira ordem, com a constante de velocidade de 2,33 x 10^{-1} µg ml^{-1} h^{-1}, meia-vida de 3,0h e t_{90} calculado igual a 4.5× 10^{-2} h (Figura 3 do ARTIGO 1).

O teste de degradação forçada foi realizado para demonstrar a especificidade do método por cromatografia eletrocinética micelar. Nas condições de estresse hidrolíticas, as soluções amostra foram tratadas com HCl 0,1 M durante 3 h e NaOH 0,5 M durante 6 h. A degradação ácida mostrou um decréscimo de 13% na área do pico de dronedarona e produziu um novo pico. Em condições alcalinas, o decréscimo foi de 17,4% e um novo pico foi detectado em 4,5 min. A degradação oxidativa com peróxido de hidrogênio a 25% por 5 h resultou em um decréscimo de 24% na área do pico principal e um novo pico foi detectado com tempo de retenção de 4,76 min. Não foram detectados produtos de degradação nas condições fotolítica e térmica, embora houvesse redução da área do pico da dronedarona de 55% e 13%, respectivamente. A partir dos resultados experimentais obtidos nos testes de degradação forçada realizados, através dos dois métodos analíticos, observa-se que a dronedarona é instável nas condições de hidrólise ácida, hidrólise básica e oxidação, onde houve formação de produtos de degradação. Na condição fotolítica houve degradação, entretanto, nenhum pico foi detectado, sugerindo a formação de produto de degradação que não contém grupos cromóforos ou que não possuem condições de migração.

7.6 Método espectrofotométrico aplicado no estudo de dissolução de dronedarona em comprimidos revestidos

7.6.1 Dissolução

A absorção de um fármaco, a partir de uma forma farmacêutica sólida, após a administração oral, depende da liberação dessa substância a partir do produto farmacêutico, da dissolução e da solubilização do fármaco sob condições fisiológicas e da permeabilidade através do trato gastrintestinal. Devido à natureza crítica das duas primeiras etapas, a dissolução *in vitro* pode ser relevante para a predição do desempenho *in vivo* (FDA,1997). Os estudos de dissolução *in vitro* constituem uma ferramenta importante para a caracterização da qualidade biofarmacêutica da forma farmacêutica de uso oral nas várias etapas dos processos de desenvolvimento galênico, na identificação das variáveis críticas na produção, formulação e controle de qualidade (MANADAS et al., 2002). O desenvolvimento de testes de dissolução para compostos pouco solúveis em água em formas farmacêuticas sólidas de liberação imediata apresenta muitos desafios. Esses desafios incluem o desenvolvimento e a validação

do teste de dissolução, assegurando que o método é discriminatório além de abordar o estabelecimento de correlações *in vitro/ in vivo* (BROWN, 2004).

Não há teste de dissolução descrito na literatura para dronedarona em comprimidos, nesse sentido foi desenvolvido e otimizado um teste de dissolução para estudar a liberação da dronedarona a partir da forma farmacêutica, conforme demonstrado no **ARTIGO 3**. A solubilidade é um parâmetro de grande relevância na escolha do meio de dissolução (BROWN, 2004). A solubilidade da dronedarona foi determinada em diferentes meios: tampão acetato de sódio, ácido clorídrico 0,1 M, lauril sulfato de sódio, tampão fosfato de potássio, polissorbato 20 e polissorbato 80 (Figura 2). Os resultados obtidos em HCl 0,1M, lauril sulfato de sódio e tampão fosfato de potássio pH 6,8 demonstraram que a solubilidade nesses meios é baixa e, portanto, esses meios foram descartados para o teste de dissolução. Na presença do tampão acetato de sódio pH 4,5, a solubilidade aumenta para 2,02 mg/mL. A maior solubilidade foi obtida com o uso de polissorbato 80 no meio de dissolução a 2,0% (2,10 mg/mL; com 1,0% a solubilidade foi de 1,88 mg/mL).

Os comprimidos revestidos contendo dronedarona foram testados em 900 mL de tampão acetato de sódio (pH 4,0 e 4,5), tampão fosfato de potássio (pH 4,0 e 4,5) e polissorbato 80 nas concentrações de 1,0 e 2,0% (Figura 3). A maior velocidade de liberação foi obtida com o meio polissorbato 80 a 2,0%, entretanto, devido a maior dificuldade de dispersão desse tensoativo em água na concentração de 2,0%, optou-se por utilizá-lo na concentração de 1,0%. Com o uso do tampão acetato pH 4,5, a taxa de liberação da dronedarona foi maior em relação ao meio contendo polissorbato a 1,0%, por isso estes dois meios de dissolução foram testados e comparados. As condições para o teste de dissolução foram otimizadas utilizando-se o aparato pá com velocidades de agitação de 50 e 75 rpm, as quais são normalmente utilizadas para o teste de dissolução de comprimidos (USP 34, 2011). Com base nos perfis de dissolução obtidos, observa-se que com o aumento da velocidade de agitação há o aumento da taxa de liberação do fármaco, por isso a velocidade de 75 rpm foi escolhida.

O perfil de dissolução utilizando o tampão acetato pH 4,5 como meio de dissolução foi comparado com o perfil obtido com o meio polissorbato 80 a 1% (Figura 4). Na comparação dos dois perfis foi utilizado o método modelo independente simples, empregando um fator de diferença (f1) e um fator de similaridade (f2). A partir dos resultados obtidos através do cálculo dos fatores f1 e f2, os dois perfis de dissolução seriam considerados semelhantes, pois atendem aos critérios exigidos pelos dois fatores. Os dois perfis também foram avaliados através da eficiência de dissolução (ED) (Tabela 1), que se define com a área sob a curva de

dissolução até um determinado tempo *t* e exprime-se como uma percentagem da área do retângulo correspondente a 100% da dissolução do mesmo período de tempo. No entanto, de acordo com os resultados obtidos para a ED % para os dois perfis nos tempos de 60, 75 e 90 minutos, que foram submetidos à análise de variância, a porcentagem do fármaco liberado não é similar entre os dois meios de dissolução. Os valores de ED% foram mais altos para o meio de dissolução contendo tampão acetato pH 4,5 (65,83% ± 3,9) do que o valor obtido para o polissorbato 80 a 1,0% (58,59 ± 3,2).

De acordo com a literatura, as formas farmacêuticas orais de liberação imediata devem liberar 85% do fármaco entre os 15 e 60 minutos (FDA, 1997). Com o uso do tampão acetato pH 4,5 como meio de dissolução e velocidade de agitação de 75 rpm, a liberação de dronedarona foi de 89,68% em 60 minutos (Figura 4), a qual está de acordo com a especificação preconizada pelo FDA (1997). Entretanto, a liberação obtida utilizando o meio polissorbato 80 a 1,0% a 75 rpm foi de 78,33% em 60 minutos (Figura 4).

A dronedarona é um composto com baixa solubilidade em água. Para os compostos pouco solúveis em água, a principal limitação para a absorção oral é a velocidade de dissolução, porque não há fluidos gastrintestinais suficientes para dissolver a dose administrada (DRESSMAN et al., 1998; DRESSMAN; REPPAS, 2000). A dronedarona deve ser administrada duas vezes ao dia concomitantemente com as refeições (EMA, 2012). O meio de dissolução utilizado para mimetizar as condições fisiológicas do trato gastrintestinal no estado alimentado (considerando-se que no duodeno o valor do pH é próximo de 5,0) contém um tampão acetato devido ao fato de que essa simulação deve ser feita com uma alta capacidade tamponante mantendo o valor de pH baixo. O volume de até 1L representa as secreções gástricas induzidas pela refeição (DRESSMAN et al., 1998).

Considerando-se os fatores mencionados anteriormente, as condições escolhidas para o teste de dissolução foram as seguintes: 900 mL de tampão acetato pH 4,5, utilizando o aparato pá a uma velocidade de agitação de 75 rpm no tempo de 90 minutos. Nessas condições, o método espectrofotométrico foi validado utilizando o tampão acetato pH 4,5 como diluente para avaliar a porcentagem de dissolução de comprimidos de dronedarona.

7.6.2 Método espectrofotométrico

O segundo método espectrofotométrico foi desenvolvido e validado para ser aplicado nos estudos de dissolução *in vitro* de comprimidos revestidos contendo dronedarona, conforme demonstrado no **ARTIGO 3**. Entre as vantagens desse método para a determinação de dronedarona em forma farmacêutica destaca-se o diluente utilizado (tampão acetato de sódio pH 4,5), que apresenta uma toxicidade mais baixa, além de reduzir o uso e descarte de solventes orgânicos e, por consequência, os custos de análise. A especificidade do método foi avaliada através da análise do espectro do placebo, comparando-o com o espectro da solução amostra após 90 minutos do teste de dissolução (Figura 5). A partir do espectro obtido para o placebo, onde não houve absorção nos comprimentos de absorção máxima da dronedarona, demonstrando não haver interferência dos excipientes, constatou-se que o método é específico. O método foi linear no intervalo de concentrações entre 2,5 a 25,0 µg/mL, obtendo-se a seguinte equação da reta: y = 0,0315x – 0,0117 (r = 0,9998). Os resultados obtidos com as três curvas analíticas foram submetidos à análise estatística por ANOVA, que indicou regressão significativa e ausência de desvio da linearidade ($p < 0,05$). A precisão do método foi avaliada através da repetibilidade e precisão intermediária (Tabela 2). O resultado médio obtido na repetibilidade foi de 99,60% com DPR de 0,21% (n = 6). Na precisão intermediária, o DPR obtido para a precisão entre dias foi de 0,25% (n = 12) e o DPR entre analistas foi de 0,31% (n = 12). A exatidão foi calculada pela porcentagem de recuperação, obtendo-se valores médios entre 98,0 a 101,8% (99,9 ± 1,13%, média ± DPR, n = 9) (Tabela 3). Esses valores se encontram dentro do intervalo recomendado para a exatidão de métodos analíticos (98,0 a 102,0%). A robustez do método espectrofotométrico foi estudada através da análise de amostras em diferentes comprimentos de onda e diferentes valores de pH do tampão acetato (Tabela 4). Essas pequenas variações nos parâmetros analíticos não conduziram a diferenças significativas na determinação de dronedarona em forma farmacêutica, sugerindo a robustez do método. Além disso, foi estudada a estabilidade da dronedarona em tampão acetato pH 4,5 por 24 h em temperatura ambiente e o resultado do teor obtido se manteve em 100,85 ± 1,4%. O método espectrofotométrico proposto mostrou-se adequado tanto para o estudo de dissolução dos comprimidos *in vitro* como para o controle de qualidade de comprimidos de dronedarona.

CONCLUSÕES

8 CONCLUSÕES

- Os métodos por cromatografia líquida em fase reversa com detecção UV, espectrofotometria no UV e eletroforese capilar utilizando a técnica de cromatografia eletrocinética micelar, desenvolvidos e validados, mostraram-se específicos, sensíveis, lineares, precisos, robustos e exatos, sendo adequados para a quantificação de dronedarona em comprimidos revestidos, podendo ser aplicados em análises de rotina e em estudos de estabilidade de dronedarona.

- A análise comparativa dos métodos por cromatografia líquida, espectrofotometria no UV e eletroforese capilar demonstrou não haver diferença significativa entre eles ($p > 0,05$), comprovando a intercambialidade dos métodos.

- O estudo de degradação forçada, realizado nas amostras de comprimidos de dronedarona, demonstrou a formação de produtos de degradação nas condições de hidrólise ácida e básica e oxidação, sugerindo a suscetibilidade do fármaco a essas condições de estresse. Não foram detectados produtos de degradação nas condições fotolítica e térmica.

- O método validado por espectrofotometria no UV utilizando o tampão acetato pH 4,5 como diluente foi adotado para avaliar a percentagem dissolvida dos comprimidos de dronedarona. O teste de dissolução foi realizado com o uso de 900 mL de tampão acetato pH 4,5 a 37°C, aparato pá com velocidade de agitação de 75 rpm, demonstrando resultado satisfatório para a análise da dissolução de comprimidos contendo dronedarona.

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