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ARAŞTIRMA MAKALESİ /**RESEARCH ARTICLE**

DIRECT FLOW INJECTION ANALYSIS ASSAY FOR THE DETERMINATION OF AMIODARONE IN PHARMACEUTICAL FORMULATIONS

Sinem ILGIN [1], Bülent ERGUN [1], Göksel ALTIOKKA [2]

ABSTRACT

Amiodarone hydrochloride (2-butyl-3-benzofuranyl-4-[2(diethylamino) ethoxy]-3,5 diodophenyl ketone hydrochloride) is a class III anti-arrhythmic agent and one of the most powerful drugs used in the treatment of ventricular and supraventricular tachycardias. In the present study, a flow injection analysis of amiodarone hydrochloride using UV detection is described. The best solvent system was found as %10 methanol. A flow rate of 1.4 ml.min^{-1} was pumped and active material was detected at 242 nm. The calibration equation was linear in the range of 5.54x10^{-8} and 2.70x10^{-7} M. Limit of detection and limit of quantification were calculated 1.84x10^{-8} and 5.54x10^{-9} M. The proposed method was applied to the determination of AMI in the pharmaceutical preparations. The results were compared with those obtained from UV-Spectrophotometry. The results showed that there is a good agreement between flow injection analysis method and UV-Spectrophotometry. The validation studies were realized by the related applications and the results were evaluated statistically. According to the results, non-significant difference was observed between the methods.

Keywords: Amiodarone, Flow injection analysis, UV-Spectrophotometry.

FARMASÖTİK FORMÜLASYONLARDA AMİODARON TAYİNİ İÇİN DİREKT AKIŞ ENJEKSİYON ANALİZ YÖNTEMİ

ÖZ

Amiodaron hidroklorid (2-bütil-3-benzofuranil-4-[2(dietilamino)etoksi]-3,5 diiyodofenil keton hidroklorid) sınıf III antiaritmik bir ajan olup ventriküler ve supraventriküler taşikardinin tedavisinde kullanılan en güçlü ilaçlardan biridir. Bu çalışmada amiodaron hidroklorid için UV deteksiyon kullanılarak bir akış enjeksiyon analizi tanımlanmıştır. Amiodaron hidroklorid için en iyi çözücü sistemi %10 metanol olarak belirlenmiştir. 1.4 ml.dk^{-1} akış hızı kullanılmış ve aktif materyal 242 nm.'de detekte edilmiştir. Kalibrasyon denklemi 5.54x10^{-8} ve 2.70x10^{-7} M aralığında doğrusal olarak bulunmuştur. Tayin ve saptama sınırı sırasıyla 1.84x10^{-8} ve 5.54x10^{-9} M olarak hesaplanmıştır. Önerilen metot amiodaron içeren farmasötik preparatlara uygulanmıştır. Sonuçlar UV spektrofotometrisinden elde edilen sonuçlarla karşılaştırılmıştır. Sonuçlar akış enjeksiyon analizi ve UV spektrofotometrisi arasında iyi bir uyumluluk olduğunu göstermiştir. Validasyon çalışmaları ilişkili uygulamalarla gösterilmiş ve sonuçlar istatistiksel olarak değerlendirilmiştir. Bu sonuçlara göre metotlar arasında farkın istatistiksel olarak önemsiz olduğu görülmüştür.

Anahtar Kelimeler: Amiodaron, Akış enjeksiyon analizi, UV-Spektrofotometrisi.

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1. INTRODUCTION

Amiodarone (AMI) is a class III anti arrhythmic agent. It is used the for treatment of many ventricular and supraventricular arrhythmias including atrial fibrilation (Weir and Ueda, 1986; Jun and Brocks, 2001; Hanioka et al., 2002; Perez-Ruiz et al., 2006; Maes et al., 2006). It is a white powder derived from benzofuran, and has a molecular weight of 645.32, ($C_{25}H_{29}I_2NO_3$). It is usually presented as a hydrochloride (MW 681.8) (Enna, 2007); which is not very soluble in water, and very lipophilic (Hanioka et al., 2002). (Figure 1)

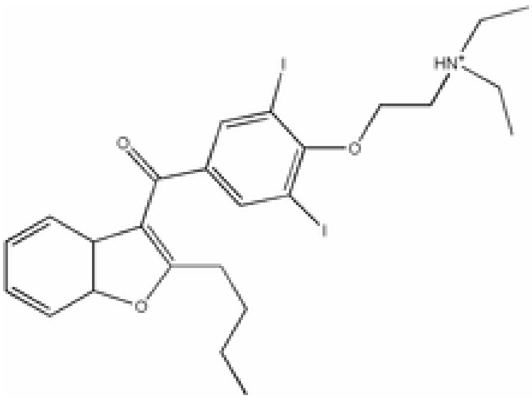

Figure 1. The chemical structure of AMI

The bioavailability of AMI is variable but generally poor, ranging from 22 to 95 percent. AMI is sequestrated in high concentrations in fat and muscle, as well as in the liver, lungs, and skin. The major metabolite of AMI is desethylamiodarone (DEA), which is known to have antiarrhythmic properties. The elimination half-life of AMI is highly variable and unusually long, averaging about 58 days (Podrid, 1995; Jun and Brocks, 2001; Siddoway, 2003).

AMI has been associated with toxicity involving the lungs, thyroid gland, liver, eyes, skin, and nerves (Campbell and Williams, 1998; Siddoway, 2003; Perez-Ruiz et al., 2008). Although as many as 80% of patients have some side effects, only 10% to 15% of patients require withdrawal of the drug because of serious or disturbing toxicity. (Ha et al., 2001). The frequency of most adverse effects is related to the total AMI exposure (i.e., dosage and duration of treatment). Therefore, physicians must use the lowest possible dosage of AMI and, if possible, discontinue treatment whenever adverse effects occur (Campbell and Williams, 1998; Siddoway, 2003; Perez-Ruiz et al., 2008).

AMI is a potent inhibitor of the hepatic and renal metabolism of several drugs. AMI inhibits metabolism through several cytochrome P450 pathways, including CYP 2C9 (which metabolizes warfarin), CYP 2D6 (which metabolizes several beta blockers and narcotics), and CYP 3A4 (which metabolizes cyclosporine and calcium channel blockers) (Podrid, 1995; Siddoway, 2003).

Despite potential adverse effects, clinical use of AMI is increasing because of its efficacy in treating arrhythmias. Thus there is a continued need for a rapid, practical AMI assay to study the relationship between serum concentrations and clinical effects and to guide safer dosing (Perez-Ruiz et al., 2006).

Several methods have been developed for the determination of AMI in different matrices. For the analysis of biological fluids, high performance liquid chromatographic and capillary electrophoretic methods have often been proposed for the determination of AMI (Perez-Ruiz et al, 2006, Perez-Ruiz et al, 2008). For the determination of this drug in pharmaceutical preparations, potentiometric titration, spectrophotometry, infrarred spectroscopy, fluorimetry, liquid chromatography and electrochemical methods have been reported (Perez-Ruiz et al., 2006).

Flow-injection analysis (FIA) has a great importance in the latest years. Since it is fast, cheap, accurate and precise, it has very widely applications in many areas (Altıokka et al., 2001; Can et al., 2008).

This study describes a simple, rapid, sensitive and practical method for the determination of AMI in pharmaceutical formulations. The optimum parameters were investigated and method validation studies were performed.

2. EXPERIMENTAL

2.1 Chemicals

The standard AMI was supplied from Sanofi-Syhthelabo Ilac A.S. (Istanbul, Turkey). Other chemicals were of analytical grade and provided from Merck Com. (Darmstadt, Ge). The commercial preparation of AMI, Cordarone® ampule (each containing 150 mg AMI, Sanofi-Syhthelabo Ilac A.S. (Istanbul, Turkey)) and tablet (each containing 200 mg AMI, Sanofi-Syhthelabo Ilac A.Ş. (Istanbul, Turkey)) were purchased from a local pharmacy.

2.2 Apparatus

The FIA system consisting of a Model Spectra System SCM 1000 degasser, Spectra System P1000 isocratic pump, Spectra System SN4000 connector, Spectra System UV6000LP diode array detector (Thermo Finnigan, USA).

Spectrophotometric studies were conducted using UV-2401 PC Spectrophotometer. (Shimadzu, Japan).

2.3 Procedures

Preparation of solutions

Standard AMI solution was prepared by dissolving it in 50 ml of methanol. This stock solution ($5.54x10^{-4}$ M) was employed for the preparation of other dilutions. Mobile phase was an aqueous solution of methanol (10%, v/v).

For the quantification of AMI, 1 Cordarone® tablet consisting of 200 mg AMI was dissolved in methanol. It was sonicated for 30 minutes and it was made up to 100 ml by methanol.

For the quantification of AMI, 1 Cordarone® ampule consisting of 150 mg AMI was dissolved in methanol. It was sonicated for 30 minutes and it was made up to 100 ml by methanol.

3. RESULTS AND DISCUSSION

3.1 Optimization of FIA Method

Analytical parameters were considered compromising the component of the solvent system. Solvent system must dissolve AMI, it must be cheap and easily provided. It was decided that 10 percent methanol is very suitable solvent for these purpose.

A 1.11x10-6 M AMI solution was prepared, diluting with methanol from the stock solution. The spectrum of the solution was recorded in the wavelength range of 200-400 nm. A maximum appeared at 242 nm. This wavelength is very available because it is highly far from UV region.

The effect of flow-rate on the peak area and peak height of AMI ($5.54x10^{-8}$ M) in the range of 0.5-1.5 ml.min-1 was investigated. Bigger peak areas and skewed peaks were appeared by pumping through flow-rate values lower than 1.0 ml.min^{-1}. Asymmetric peaks with lower peak areas were observed with a flow-rate higher than 1.4 ml.min-1. (Table 1.). Suitable flow rate was chosen as 1.4 ml.min-1 to perform the quantification studies and peak area response was used, in the rest of experiments.

3.2 Repeatability and Intermediate Precision

Repeatability and intermediate precision were tested using $1.66x10^{-7}$ M AMI solution in methanol at 1.4 ml.min-1 of flow-rate and 242 nm of detection wavelength in three operating days with 8 samples. The results were evaluated using the response of AMI involving peak area and peak height as can be seen in Table II. Very low variation coefficients below 2% of relative standard deviation (RSD) were obtained showing the method is sufficiently precise. (Table 2.)

3.3 Linearity

The calibration line of response of peak area as a function of the concentration was constructed in the concentration range of $5.54x10^{-8}$–$2.70x10^{-7}$ M of AMI solution at three operating days. The detailed statistical results are shown in Table III. (Table 3.) Statistically evaluated data show acceptable linearity with high regression coefficients and intercepts close to the origin, in the studied range for FIA. (Figure 2.)

3.4 Detection and Determination Limits

The detection limit of the FIA method was found to be 1.84x10-8 M according to the criteria of signal-to-noise (S/N = 3) and the limit of quantification (LOQ) was calculated to be $5.54x10^{-9}$ M accepting the signal-to-noise (S/N = 10).

3.5 Application of the Method to the Commercial Tablet and Ampule

Commercial AMI tablets are produced containing 200 mg AMI. The developed method for the determination of AMI was applied to the commercial tablets employing optimum FIA conditions. And these conditions were also applied to AMI ampule containing 150 mg AMI.

The accuracy expresses the closeness of agreement between the value found and the value that is accepted as a reference value. A standard method, UV-spectrophotometry, was used to compare with the method accuracy of flow-injection analysis.

Table 1. Flow Rate

Flow Rate	Mean	SD	%RSD
0.5	193934.67	3377.28	1.74
0.7	242329.00	3203.00	1.32
0.8	256910.67	2578.46	1.00
1	283704.33	2998.97	1.06
1.2	300281.00	2482.71	0.83
1.4	323403.33	731.87	0.23
1.5	356587.67	2814.97	0.79

Table 2. The repeatability and intermediate precision tests of AMI (1.66x10^{-7} M)

	Repeatability			Intermediate Precision (n=24)
	Day 1 (n=8)	Day 2 (n=8)	Day 3 (n=8)	
	Area	Area	Area	Area
Mean	502935.8	503293.8	503156.6	503128.7
SD	1261.99	1609.54	1695.36	1428.19
RSD %	0.25	0.32	0.34	0.28

Table 3. The linearity results of AMI peak area signals in the concentration range of 5.54x10^{-8} – 2.70x10^{-7} M with 1.4 ml.min-1 flow-rate and at 242 nm detection wavelength.

	Intra-day			Inter-day
	Day 1 (n=8)	Day 2 (n=8)	Day 3 (n=8)	Whole Days (n=24)
Slope	1.63x10^{12}	1.62x10^{12}	1.62x10^{12}	1.62x10^{12}
Intercept	228578.03	232089.00	232015.93	230894.32
Correlation Coefficient	0.9998	0.9996	0.9997	0.9996

UV-spectrophotometry was used as comparison method. A good linear relation between absorbance and concentration of AMI was obtained in the range of 5.40x10^{-7}-4.44x10^{-6} M at 242 nm of detection wavelength using methanol as blank. It fits to the equation of [A = 331000.8C (M) - 0,0184; r = 0.9993].

The results of the commercial tablet and ampule assayed by FIA and UV spectrophotometry as described in the experimental section are presented in Table 4 and Table 5. (Table 4 and Table 5)

The results of statistical analysis show no significant difference between the proposed method and standard method. As a conclusion the method proposed in this study is simple, accurate, precise and rapid. Therefore, the suggested method is more practical, regarding the time of analysis, consumption of solvents and size of sample required fort the routine analysis of AMI.

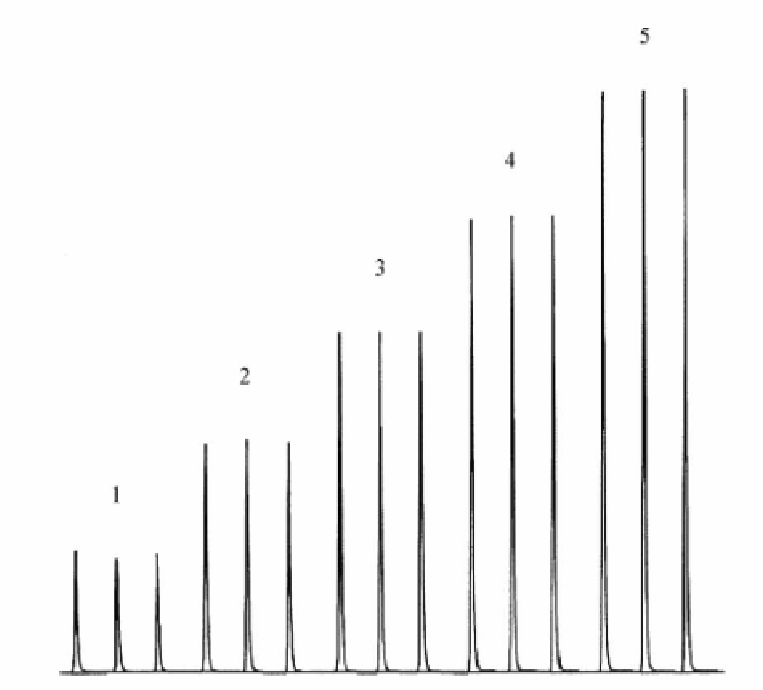

Figure 2. UV signal concentration of AMI (1; $5.54x10^{-8}$ - 2; $1.11x10^{-7}$ - 3; $1.66x10^{-7}$ - 4; $2.22x10^{-7}$ - 5; $2.70x10^{-7}$ M)

Table 4. The determination results of AMI in commercial tablet (Declared amounts of tablet = 200 mg)

	FIA	UV
Mean (n=8)	202.72	201.45
SD	0.59	0.49
RSD %	0.29	0.24

Table 5. The determination results of AMI in commercial ampule (Declared amounts of ampule = 150 mg)

	FIA	UV
Mean (n=8)	150.39	148.22
SD	0.35	0.28
RSD %	0.23	0.19

REFERENCES

Altıokka, G. and Atkoşar, Z. (2001). Flow Injection Analysis of Doxazosin Mesylate Using UV-detection. *J. Pharm. Biomed. Anal* 27, 841-844.

Campbell, T.J. and Williams, K.M. (1998). Therapeutic drug monitoring: Antiarrhythmic drugs. *Br. J. Clin. Pharmacol* 46, 307–319.

Can, N.O., Altiokka, G. and Aboul-Enein, H.Y. (2008). Determination of ticlopidine in pharmaceutical tablets by flow injection analysis using UV-detection. *J. Liq. Chrom. Rel. Technol*. 31, 3209-3218.

Enna, S.J. and Bylund, D.B. (2008). Amiodarone. xPharm: The Comprehensive Pharmacology Reference, 1-2.

Ha, H.R., Bigler, L., Binder, M., Kozlik, P., Stieger, B., Hesse, M., Altorfer, H.R. and Follath, F. (2001). Metabolism of Amiodarone (Part I): Identification of a new hydroxylated metabolite of amiodarone. *J. Exp. Phar. Ther*. 29(2), 152–158.

Hanioka, N., Saito, Y., Soyama, A., Ando, M., Ozawa, S. and Sawada, J. (2002). High-performance liquid chromatographic assay for amiodarone N-deethylation activity in human liver microsomes using solid-phase extraction. *J. Chromatogr. B* 774, 105–113.

Jun, A.S. and Brocks, D.R. (2001). High-Performance liquid chromatographic assay of amiodarone in rat. *J. Pharm. Pharmaceut. Sci.* 4(3), 263-268.

Maes, A., Baert, K., Croubels, S., De Clercq, D., van Loon, G., Deprez, P. and De Backer, P. (2006). Determination of amiodarone and desethylamiodarone in horse plasma and urine by high performance liquid chromatography combined with UV detection and electrospray ionization mass spectrometry. *J. Chromatogr*. B 836, 47–56.

Perez-Ruiz, T., Martinez-Lozano, C. and Garcia-Martinez, M.D. (2008). Simultaneous determination of amiodarone and its metabolite desethylamiodarone by high-performance liquid chromatography with chemiluminescent detection. *Analytica Chemica Acta* 623, 89–95.

Perez-Ruiz, T., Martinez-Lozano, C., Martın, J. and Ruiz, E. (2006). Flow injection chemiluminescent determination of amiodarone in pharmaceutical preparations using photogenerated tris(2,2_-bipyridyl)ruthenium(III). *J. Pharm. Biomed. Anal*. 42, 143–147.

Podrid, P.J. (1995). Amiodarone: Reevaluation of an old drug, diagnosis and treatment. *Ann. Intern. Med.* 122(9), 689-700.

Siddoway, L.A. (2003). Amiodarone: Guidelines for use and monitoring. Am. Fam. Physician 68, 2189-2196.

Weir, S.J. and Ueda, C.T. (1986). Amiodarone Pharmacokinetics. I. Acute dose-dependent disposition studies in rats. J. Pharmacokinet. Biopharm 14(6), 601-613.

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