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QUANTITATIVE DETERMINATION OF AMLODIPINE CONTAINING PHARMACEUTICALS BY IR SPECTROSCOPY AND HPLC METHODS

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SUMMARY

In this study, IR and HPLC methods are described for the quantitative determination of amlodipine (AMP) in a solid dosage form.

IR spectroscopic method (KBr disc) was used and disulfiram (DSF) was chosen as the internal standard. The specific absorption bands at 693 cm^{-1} and 913 cm^{-1} were chosen for AMP and DSF respectively. For AMP Beer Law was obeyed in the concentration range of 0.6-2 % w/w in KBr. At 693 cm^{-1} regression equation was found to be y=0.652x+0.024 (r^2=0.9968). The mean recovery and RSD values were found to be 98.2 % and 1.63 % for IR spectroscopic method, respectively.

In HPLC method good chromatographic separation was achieved using a Luna C$_{18}$ 5μm (250x4.6 mm) column and a mobile phase consisting of water-methanol-phosphate buffer (36:64:1 v/v) at a flow-rate of 0.7 mL.min^{-1}. Mefrusid (MFD) was chosen as the internal standard. AMP and MFD were detected at 275 nm and eluted at 11.5 and 7.27 min. respectively. Linearity range for AMP was 0.004-0.020 mg.mL^{-1}. The regression equation was found to be y=0.846x-0.013 (r^2=0.9950). The mean recovery and RSD values were found to be 98.9 % and 0.75 % respectively.

ÖZET

Bu çalışmada , Amlodipin (AMP) içeren katı dozaj formundaki müstahzarda AMP nin IR spektroskopisi ve Yüksek Basınçlı Sıvı Kromatografisi yöntemleri ile kantitatif tayini gerçekleştirilmiştir. IR spektroskopisi yönteminde KBr tablet tekniği kullanılmış, disulfiram (DSF) internal standart olarak seçilmiştir. Çalışmalarda spesifik absorpsiyon bantları olarak AMP ve DSF için sırası ile 693 cm^{-1} ve 913 cm^{-1} seçilmiştir. AMP için Beer yasasına göre uygun konsantrasyon aralığı % 0.6-2 a/a'dır. 693 cm^{-1} de saptanan regresyon denklemi y=0.652x+0.024 (r^2=0.9968)'dir. Ortalama geri kazanım değeri ve relatif standart sapma değerleri IR spektroskopisi yöntemi için sırası ile % 98.2 ve % 1.63 olarak bulunmuştur.

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Yüksek Basınçlı Sıvı Kromatografisi yönteminde kolon sistemi olarak Luna C₁₈ 5μm (250x4.6 mm) kullanılmış, hareketli faz olarak su-metanol-fosfat tamponu (pH 3) (36:64:1) sistemi seçilmiştir. Akış hızı dakikada 0.7 mL'dir. İnternal standart olarak Mefrusid (MFD) seçilmiştir. Ölçümler 275 nm'de yapılmış ve pikler AMP ve MFD için sırası ile 11.5 ve 7.27 dakikalarda gözlenmiştir. AMP için lineer konsantrasyon aralığı 0.004-0.020 mg.mL⁻¹dir. Regresyon denklemi y=0.846x-0.013 (r²=0.9950) olup ortalama geri kazanım değeri ve bağıl standart sapma değeri sırası ile % 98.9 ve % 0.75 olarak bulunmuştur.

Key words: Amlodipine, IR Spectroscopy, HPLC, Quantitative Determination

INTRODUCTION

The main use of calcium-channel blockers is in the management of angina pectoris and hypertension. Some are also employed in cardiac arrhythmias. Amlodipine (AMP) [2-[2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl-5-methyl ester (Fig-1) and other dihydropyridine derivatives are used for their antihypertensive and antianginal properties (1,2)
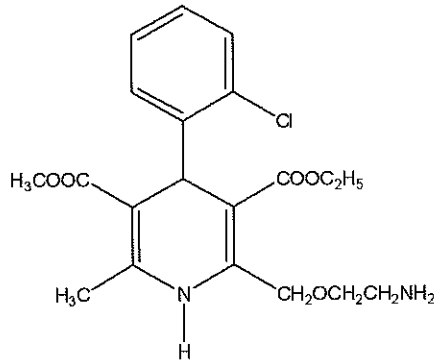

Figure 1. Amlodipine

In previous studies High Performance Thin Layer Chromatographic (3), spectrophotometric (4-6) , derivative Spectrophotometric (7,8), Differential-pulse Voltametric (9), High Pressure Liquid Chromatographic (10-14), GC-Mass Spectrometric (15), LC-Mass Spectrometric methods(16,17) were reported for the quantitative determination of AMP in body fluids and pharmaceutical dosage forms. Hitherto there is no IR spectroscopic method reported for the quantitative determination of AMP in a solid dosage form.

In this study IR and HPLC methods are described for quantitative determination of AMP in a solid dosage form.

RESULTS AND DISCUSSION

IR spectroscopy is an analytical method used mostly for the structural elucidation and purity control of chemical substances. Less frequently it is used for the quantitative determination of drugs (18-20).
In this study, disc technique was used, and absorption bands at 693 cm⁻¹ and 913 cm⁻¹ were chosen for AMP and DSF respectively (Fig-2).
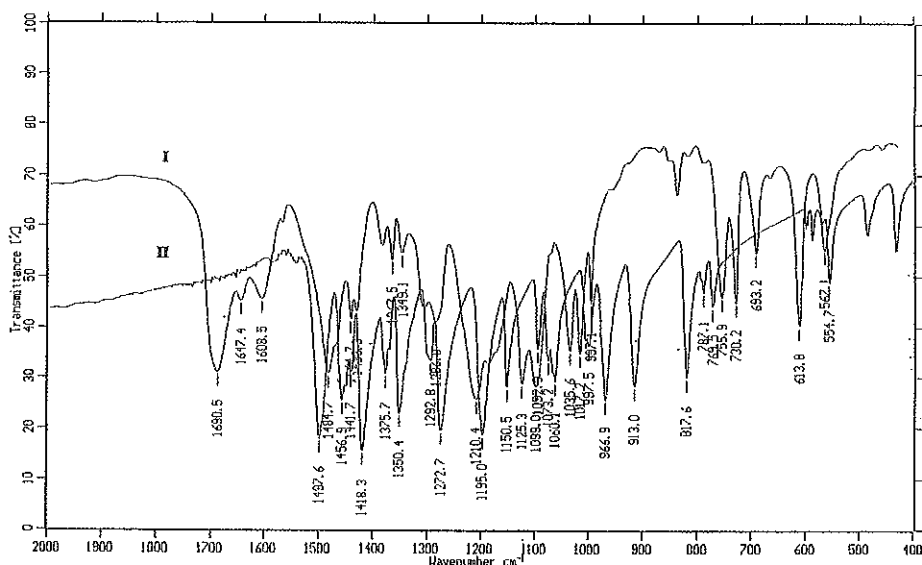

Figure 2. Infrated Spectrum of AMP and DSF in KBr
I:AMP II:DSF (Int.St.)

Internal standard was used in order to eliminate some errors that might originate from the application of the method. For this purpose, DSF was especially chosen as the internal standard with the absorption band at 913 cm⁻¹ where AMP has no absorption. On the other hand DSF has no absorption band at 693 cm⁻¹ where AMP has absorption (Fig-3).

Quantitative determination is based on the concentration-absorption relationship of Beer's Law. P_B and P_o points of the absorption peaks are assigned with the base-line technique. The regression equation was constructed using AMP/DSF concentration
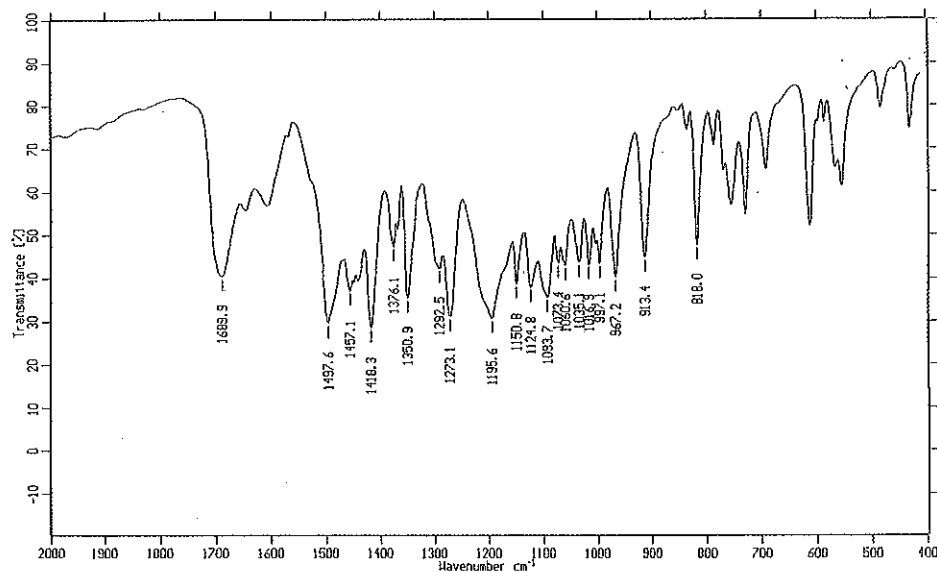
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Figure 3. IR Spectrum of AMP-DSF mixture in KBr

ratio as (x) values and the ratio of Log P_B-Log P_0 of AMP and Log P_B-Log P_0 of DSF as (y) values. At 693 cm^{-1}, regression was found to be y=0.652x+0.024 (r^2=0.9968) (Table-1 and 2).

Table 1. Log P_B-LogP$_0$ values and ratios found for AMP-DSF in synthetic mixtures.

SSM	Disc Weight mg	AMP (693 cm^{-1})		DSF (913 cm^{-1})		x=C_{AMP}/C_{DSF}	y=T_{AMP}/T_{DSF}
		Conc. Mg	Log P_B- Log P_0	Conc. Mg	Log P_B- Log P_0		
ST$_1$	124.2	1.22	0.052	0.976	0.065	1.25	0.8
ST$_2$	125.1	1.83	0.077	0.976	0.061	1.875	1.267
ST$_3$	124.5	2.42	0.109	0.968	0.064	2.5	1.703
ST$_4$	125.4	3.03	1.124	0.967	0.060	3.13	2.066
ST$_5$	124.8	3.606	0.144	0.962	0.059	3.75	2.44

C_{AMP} : AMP conc.in KBr disc

C_{DSF} : DSF conc.in KBr disc

T_{AMP} : Log P_B-LogP$_0$ value of AMP

T_{DSF} : Log P_B-LogP$_0$ value of DSF (Int.St.)

SSM : Synthetic Standard Mixture

Table 2. Statistical analysis of calibration graphs in the determination of AMP using proposed methods.

Parameters	IR	HPLC
Linearity Range	0.6-2 %	$4\times10^{-3}\,mg\text{-}2\times10^{-2}\,mg$
Limit of Detection (LOD)	0.25 %	$2\times10^{-4}\,mg$
Limit of Quantitation (LOQ)	0.4 %	$1\times10^{-3}\,mg$
Slope (a)	0.652	0.846
Intercept (b)	0.024	-0.013
Corr. Coefficient (r^2)	0.9968	0.9950

The second procedure in this study was the application of HPLC for the determination of AMP. In order to achieve the simultaneous elution of AMP and MFD (Int.St.) peaks under isocratic conditions the mobile phase composition was optimized. AMP and MFD eluted forming a symmetrical single peak, well separated from the solvent front. The elution orders were AMP (t_r=11.5 min) and MFD (t_r=7.27 min) at the flow-rate of 0.7 mL.min^{-1} (Fig-4).
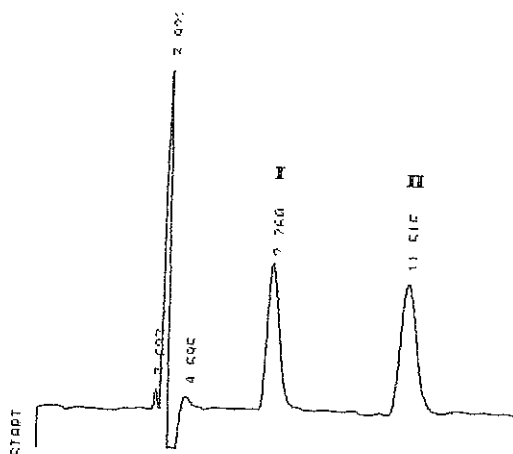

Figure 4. HPLC Chromatogram of Amlodipine and Mefrusid (Int.St.)
I.MFD (Int.St.) II.AMP

Optimum separation was realized using water-methanol-phosphate buffer (36:64:1 v/v). Detection was carried out using a UV detector at 275 nm. A linear relationship in the range of $2\text{-}12\times10^{-3}$ mg.mL^{-1} was obtained. Regression equation was found to be y=0.846x-0.013 (r^2=0.9950).

Recovery experiments were conducted to determine the accuracy of the proposed methods. The mean recovery and relative standard deviation were found to be 98.2 % and 1.63 % for IR and 98.9 % and 0.75 % for HPLC method (Table-3).

Table 3. Recovery results for the mixtures by the proposed methods.

Synthetic sample	IR			HPLC		
	Authentic		Recovery %	Authentic x10^{-3} mg.ml^{-1}		Recovery %
	Added mg	Found mg		Added mg	Found mg	
1	2.5	2.47	98.8	4	3.98	99.5
2	3.75	3.64	97.1	6	5.86	97.7
3	5	5.03	100.6	8	7.96	99.5
4	6.25	6.03	96.5	10	9.87	98.7
5	7.5	7.35	98.0	12	11.88	99.0
Mean ($\overline{X}$)			98.2			98.9
RSD %			1.63			0.75
Cofidence intervals p=0.05			$\overline{X}$±1.53			$\overline{X}$±0.71

The inter-day precision (repeatability) and accuracy were studied by repeatedly analyzing (for IR n=3 for HPLC n=5) three different concentration levels of AMP on the same day. The results are given in Table-5. The inter-day assay RSD % values were satisfactory (IR spectroscopy ~2.5, HPLC ~ 1.63) (Tables-4 and 5).

Table 4. Inter-day precision and accuracy for the determination of AMP (n=3).

Added mg %	IR Spectroscopic method				Added 10^{-3} mg.ml^{-1}	Found 10^{-3} mg.ml^{-1}	HPLC Method			
	Found mg %	Precision		Accuracy			Precision		Accuracy
		SD	RSD %	Bias*			SD	RSD %	Bias*
0.8	0.788	0.015	1.9	-1.5	6	6.11	0.084	1.37	1.8
1.2	1.166	0.037	3.17	-2.8	8	7.92	0.124	1.56	-1
1.6	1.549	0.048	3.09	-3.2	12	11.75	0.174	1.48	-2.1

*Bias= (Found-Added/Added x100)

Table 5. Assay results of commercial samples with the proposed method*
(Amlodipine tablet 10 mg)

Sample	Amount Found			
	IR		HPLC	
	Mg	Recovery (%)	Mg	Recovery (%)
1	10.09	100.9	9.95	99.5
2	9.65	96.5	9.82	98.2
3	9.82	98.2	10.06	100.6
4	10.12	101.2	10.13	101.3
5	10.25	102.5	10.04	100.4
Mean ($\overline{X}$)	99.8		100	
SD	2.44		1.19	
RSD %	2.45		1.19	
Confidence invervals p=0.05	$\overline{X}\pm2.3$		$\overline{X}\pm1.14$	

The results obtained for AMP tablets were compared with Student's t test and Fischer F test statistically. These results showed that the differences between the results of the methods were statistically insignificant.

CONCLUSION

In the literature, no IR spectroscopic method for the quantitative determination of AMP has been reported. In this study, application of the IR spectroscopic method for the determination of AMP is proposed for the first time. The suggested method can be used as an alternative method for the dosage form containing AMP as the active compound. A simple and stable isocratic HPLC assay was also developed for the analysis of AMP in commercial samples.

EXPERIMENTAL

Apparatus: IR Spectrophotometer: Brucer Vector 22 IR (Opus Spectroscopy Software, Version-2). HPLC system : Hewlett-Packard Co. Ltd. 1050 series delivery pump system equipped with UV-Vis detector. Peak areas were integrated automatically by a 3396 multimode integrator.

Reagents: Amlodipine besylate (AMP) was generously supplied by Eczacıbaşı İlaç San.ve Tic.A.Ş. İstanbul-Türkiye, Mefrusid (MFD) internal standard for HPLC was provided by Bayer İlaç Sanayi-İstanbul-Türkiye. Disulfiram (DSF) internal standard for IR was obtained from Nobel İlaç Sanayi- İstanbul-Türkiye. Methanol and water for gradient grade HPLC and Potasyum Bromide (IR Spectroscopic grade) were purchased from Merck Co.Germany and Sigma Chemicals Co-USA, respectively. Commercial tablets containing 10 mg AMP were purchased from local pharmacies in Ankara-Turkey.

Methods
IR Spectroscopic Method
Potassium Bromide Disc Technique
Stock Solution
The stock solution of AMP (2.5 mg.mL^{-1}) and stock solution of DSF (2 mg.mL^{-1}) were prepared in methanol. These solutions were stable for a week at 4°C.

Calibration Procedure
1-1.5-2-2.5-3 mL aliquots of AMP and 1 mL of DSF solutions (fixed volume) were added to 250 mg of accurately weighed potassium bromide. Methanol was evaporated under nitrogen . The remaining dry powder was mixed thoroughly with an agat pestle and a homogeneous fine powder was obtained. Discs weighing approximately 125 mg were prepared and used for the quantitative measurements.
For this purpose absorption bands at 693 cm^{-1} for AMP and 913 cm^{-1} for DSF were chosen.

Preparation of the Samples
Ten tablets were weighed and powdered in a mortar. An accurately weighed portion of the powder equivalent to about 50 mg AMP was transferred to a volumetric flask and then 20 mL was extracted with methanol for 15 min. The extract was filtered into a 25 mL volumetric flask and 50 mg DSF was added and the volume was adjusted to 25 mL with methanol. 2 mL of this solution were transferred to 250 mg accurately weighed potassium bromide in a porcelain dish. The remaining part of the procedure was continued as in KBr disc technique.

HPLC Method
Stock Solutions
The stock solutions of AMP (0.1 mg.mL^{-1}), Mefrusid (MFD) internal standard (0.1 mg.mL^{-1}) were prepared in methanol. These solutions were stable for a week at 4^{0}C.

Chromatographic Conditions
Chromatographic separation was carried out on Luna 5μ_m C$_{18}$ (250x4.6 mm) column. AMP and MFD were separated by isocratic system with a mobile phase consisting of

water-methanol-phosphate buffer (36:64:1 v/v). Phosphate buffer was adjusted to pH=3±0.1 with o-phosphoric acid.

The mobile phase was prepared daily and filtered through an 0.45 mμ membrane and degassed for 15 minutes in an ultrasonic bath before use. The flow-rate was 0.7 mL.min^{-1} and detector was set at 275 nm. The injection volumes were 20 μL. All assays were performed at ambient temperature.

Calibration Procedure

Standard solutions of AMP were prepared within the concentration range of (0.004-0.04 mg.mL^{-1}). Internal standard (MFD) concentration was chosen in range of (0.008-0.02 mg.mL^{-1}). All dilutions were prepared with methanol. 20 μL aliquots of each standard solution were injected and all applications were repeated three times. The peak area ratios of AMP to internal standard were plotted against the corresponding concentration of AMP.

Preparation of Samples

Ten tablets were weighed and powdered. A portion of a powder equivalent to about 50 mg AMP was weighed accurately, transferred into a 25 mL volumetric flask and stirred with 20 mL methanol on a magnetic stirrer for 20 minutes.The solution was filtered and diluted with methanol (concentration range 0.08 mg.mL^{-1}- 0.25 mg.mL^{-1}). 2 mL of this solution and 1 mL of internal standard solution (0.25 mg.mL^{-1}) were taken into a 25 mL volumetric flask and diluted with methanol. 20 μL of the sample solution was injected into a column.

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