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QUANTITATIVE DETERMINATION OF AMISULPRIDE IN PHARMACEUTICALS BY IR, UV SPECTROSCOPY AND HIGH PRESSURE LIQUID CHROMATOGRAPHY

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Abstract

In this study, IR, UV spectroscopic and high pressure liquid chromatographic procedures are described for quantitative determination of amisulpride (AMS) in dosage form. For IR spectroscopic method (KBr disc technique) has been used and dehydrocholic acide (DHCA) was used as internal standard. The specific absorption bands at 1057 and 1705 cm^{-1} were chosen for AMS and DHCA respectively. In this method, Beer's Law was obeyed in concentration range of 0.8-2 % w/w in KBr disc.

In HPLC study; AMS was determined by gradient system in the mobile phase consist of methanol-0.032M phosphate buffer pH: 3±0.1 (70:30v/v). Mobile phase flow rate was 0.7 $mL.min^{-1}$ and Luna C_{18} 5µ (250X4.6mm) column was used as stationary phase. Detection was carried out using a UV detector at 275 nm and atenolol (ATN) was used as internal standart.

UV spectroscopic method was also used in the quantitative determination of AMS in dosage form. 280 nm was chosen as λ_{max}. The relative standard deviations for IR, HPLC and UV spectroscopic methods were found to be 2.24, 1.79, 1.18 % respectively in synthetic standard mixtures.

***Key words**: Amisulpride, IR, HPLC, UV spectroscopy, Quantitative determination.*

Farmasötik Preparatlarda Amisulprit'in IR, UV Spektroskopisi ve Yüksek Basınçlı Sıvı Kromatografisi ile Miktar Tayini

Bu çalışmada amisulprit (AMS) içeren farmasötik preparatlarda IR, UV spektroskopisi ve yüksek basınçlı sıvı kromatografisi yöntemleri kullanılaarak AMS' in miktar tayini gerçekleştirilmiştir. IR spektroskopisi yöntemi ile yapılan çalışmada KBr disk tekniği uygulanmış ve dehidrokolik asit (DHCA) internal standart olarak seçilmiştir. AMS ve DHCA için seçilen spesifik absorbsiyon bantları sırasıyla 1057cm^{-1} ve 1705 cm^{-1}'dir. Beer yasasına uygun konsantrasyon aralığı KBr içinde % 0.8-2 a/a olarak bulunmuştur.

YBSK çalışmasında AMS gradient sistemle tayin edilmiş olup yöntemde hareketli faz olarak metanol-0.032 M fosfat tamponu (pH: 3±0.1) (70:30 h/h) seçilmiştir. Akış hızı 0.7 $mL.dakika^{-1}$ olup sabit faz olarak Luna C_{18} 5µ (250X4.6mm)kolon sistemi kullanılmıştır. Ölçümler UV dedektörde 275 nm de gerçekleştirilmiş ve internal standart olarak atenolol (ATN) kullanılmıştır.

Nicel tayinlerde uygulanan diğer bir yöntem UV spektroskopisidir. Çalışmalarda 280 nm'deki (λ_{max}) absorbans değerleri ölçülmüştür. Laboratuvarda hazırlanan standart sentetik karışımlarda IR, YBSK ve UV spektroskopisi yöntemleri ile bulunan bağıl sapma değerleri sırasıyla %2.24, 1.79, 1.18 olarak bulunmuştur.

***Anahtar Kelimeler**: Amisulprit, IR, YBSK, UV spektroskopisi, Nicel tayin.*

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Introduction

Amisulpride (AMS) (Figure 1) is a substituted benzamide derivative chemically designated as 4-amino-N-[(1-ethyl-2-pyrrolidinyl)metyl]-5-(ethylsulfonyl)-2-methoxybenzamide is mainly in a management of psychoses such as schizophrenia but also it has been tried in depression. It is antagonized dopamin D_2 and D_3 receptors and demonstrated antischizophrenic and antidysthyminic properties in man (1,2). AMS is a rasemic mixture of two enantiomer that are (-) S- and (+) R-AMS. (-) S-AMS was found to be 20-40 times more potent than (+) R-enantiomer but had about the same activity as a racemat (3).

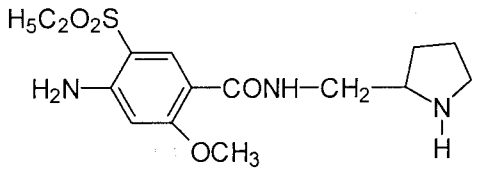

Figure 1. Structural Formula of Amisulpride.

The published methods for AMS analysis in biological materials described only using high performance liquid chromatography assay with either UV (4-6) fluorescence (7,8) or mass spectrometric detection (9). Also gas liquid chromatography assay (10) and potentiometric analysis (11) have been reported. IR spectroscopic method for the determination AMS in solid dosage form was suggested for the first time. The objectives of this study were firstly, to develop than validate two analytical methods for the quantification of AMS containing solid dosage forms.

Experimental

Apparatus

IR Spectrophotometer: Bruker Vector 22. IR (Opus Spectroscopic Software, Version-2)

HPLC system consist of Hewlett-Packard Co. Ltd 1050 series delivery pump system U_6-K universal injector 20 µL fixed loop equipped with a 1050 UV/Vis detector.

UV-Vis Spectrophotometer: Beckman DU 650 double-beam with a fixed slit width (2nm). A 1-cm quartz cell over the range 200-400 nm was employed.

Chemicals

AMS (racemat, free base, batch no: 200352) and ATN was kindly supplied from Sanofi-Doğu İlaç Sanayii, İstanbul-TURKEY.

Potassium bromide (IR spectoscopy grade) and DHCA were obtained from Sigma Chemical Company USA. Methanol and water for gradient grade HPLC were purchased from Merck-Germany. All the reagents and chemicals were of analytical grade. The phosphate buffer was prepared by adding triethylamine to potassium dihydrogen phosphate to obtain a final pH of 7±0.1. Solian^{R} tablet (Batch No: 11.00 20078.2) (produced by Sanofi-Doğu İstanbul-Turkey) containing 200 mg AMS was purchased from local pharmacies in Ankara-TURKEY.

Assay Procedure

IR Method

Potassium bromide disc technique

Stock Solutions

The stock solution of AMS (2 mg.mL^{-1}) and solution of DHCA (1 mg.mL^{-1}) were prepared in chloroform. These solutions were stable for two weeks if stored at 4 °C.

Calibration Procedure

1 - 1,5 - 2 - 2,5 - 2,5 ml of solution AMS and 1,5 - 1- 2 - 1,5 - 2 ml of solution DHCA were pipetted and poured into a 250 mg KBr powder which were weighed with a precision of 0.1 mg in a porcelain dishes separately. In this way a series of synthetic standard mixtures of AMS-DHCA (2-1,5), (3-1), (4-2), (5-1,5), (5-2) mg were quantitatively transferred into dishes separately. Chloroform was evaporated under nitrogen gas. The remaining dry powder was mixed through with an agate pestle and homogenous fine powder was obtained, after this form each mixture approximately 125 mg of discs were prepared and employed for quantitative measurement. For this purpose, 1057 cm^{-1} for AMS and 1705 cm^{-1} for DHCA absorption bands were used. The base line technique was used for the determination of P_B and P_o values of absorption bands (12). The logarithmic differences of these values as

absorbance (y) was plotted against to concentration (x) in order to calculate regression equation.

Sample Preparation

Twenty tablets were weighed powdered and a portion of powder equivalent to 150 mg AMS was accurately weighed and extracted with 35 ml chloroform. The solution was filtered into 50 ml volumetric flask and then 75 mg DHCA was added and the volume was made up to 50 ml with chloroform. 1 ml of this solution was transferred on 250 mg accurately weighed potassium bromide in porcelain disc. The remaining part of the procedure was continued as directed in calibration procedure..

HPLC Method

Chromatographic Conditions

Chromatographic separation was carried out on Luna C_{18} 5μ (250X4.6mm) column. AMS was determined by gradient system with mobile phase consisting of 0.032 M phosphate buffer-methanol (30:70) v/v, then phosphate buffer is adjusted to pH 7±0.1 with triethylamine. ATN was used as internal standard and the flow rate was 0.7 $ml.min^{-1}$. The mobile phase prepared daily and filtered through a millipore 0.45μ membran. The detector was set at 275 nm. The injection volume was 20 μL. All assay was performed at ambient temperature.

Stock Solutions

The stock solution of AMS (0.04 $mg.mL^{-1}$) and ATN (0.1 $mg.mL^{-1}$) as internal standard were prepared in methanol. These solutions were stable for a week if stored at 4°C. Calibration graphs were prepared from synthetic mixtures of AMS-ATN in methanol. Standard solutions of AMS were prepared in the concentration range of 0.64-2.00 $mcg.mL^{-1}$ and ATN concentration was fixed (10 $mcg.mL^{-1}$) for every synthetic mixture. All appropriate dilutions were prepared with methanol. 20 μL volume of synthetic mixtures were injected and all applications were repeated three times. The peak height ratios of active substances (AMS) to ATN were plotted against the corresponding concentration of AMS.

Sample Preparation

Twenty tablets were weighed on powdered. A portion of powder equivalent 40 mg AMS was weighed accurately, and was stirred with 20 ml methanol on a magnetic stirrer for 15 minutes. The solution was filtered and diluted with methanol up to 25 mL. 1 mL of this solution was taken into a 100 ml volumetric flask and completed with methanol. 2.5 mL of this solution and 3 mL internal standard (0.1 $mg.mL^{-1}$ATN) were taken into a 25 ml volumetric flask and diluted with methanol up to mark. 20 µL volume of sample solution was injected into column.

UV Spectroscopic Method

Calibration Procedure

Stock solution of AMS (0.1 mg.mL-1) was prepared in methanol. Standard solutions of AMS were prepared in the concentration range of 2-18 mcg.mL-1. Absorbance of solutions are measured at 280 nm against to methanol.

Sample preparation

A portion of powder equivalent to 25 mg AMS was weighed accurately and transferred in 50 ml volumetric flask and extracted with methanol. The extract was filtered and diluted with methanol up to 50 ml. 2 ml of this solution diluted with methanol in 100 ml volumetric flask. The absorption of solution was measured at 280 nm.

Result and Discussion

IR spectroscopy is an analytical method which is used mostly for the structural elucidation and purity control of newly synthesized compounds. Less frequently it is used for the quantitative analysis of medicinals (12-15). Some of the reason for this matter are; the absorbance of the characteristic and isolated symmetric absorption bands unable for the homogeneous distribution of active compound in KBr as a result of its low concentration and difficulties appeared in disc pressing process. In this study, disc technique was used and the absorption bands (1057 and 1705 cm^{-1}) were chosen for AMS and DHCA respectively. Specificabsorption bands at 1705 cm^{-1} and 1057 cm^{-1} were described carbonyl of keton and

ethyl sulfonyl groups respectively. (16). Internal standard was used in order to eliminate some unforeseen defaults which were originated from the application of method. For this purpose, dehydrocolic acide was especially chosen as internal standard with the absorption band at 1705 cm-1 where no absorption is available for AMS. On the other hand, internal standard has no absorption band at 1057 cm-1 where AMS has an absorption. In IR spectroscopy, the linear concentration range was obtained as 0,8-2 % w/w. This range is so narrow. Because in this study more attention was paid to hold P_B and P_o points between 80-20% as transmittance value which were used for AMS and DHCA. Especially when the P_o points is under 20% transmittance, any small error for the determination of this point fairly effect the results.

In this study, the sensitive addition of AMS and DHCA with their low concentration on KBr was realized as follows; stock solutions of AMS and DHCA were prepared in chloroform and exact volumes of these solutions were transferred into KBr powder which were weighed with a precision of 0.1 mg in a porcelain dish separately. Chloroform was evaporated under nitrogen gas. Quantitative determination is based on concentration-absorption relationship of Beer's Law. PB and Po points of the absorption peaks are assigned

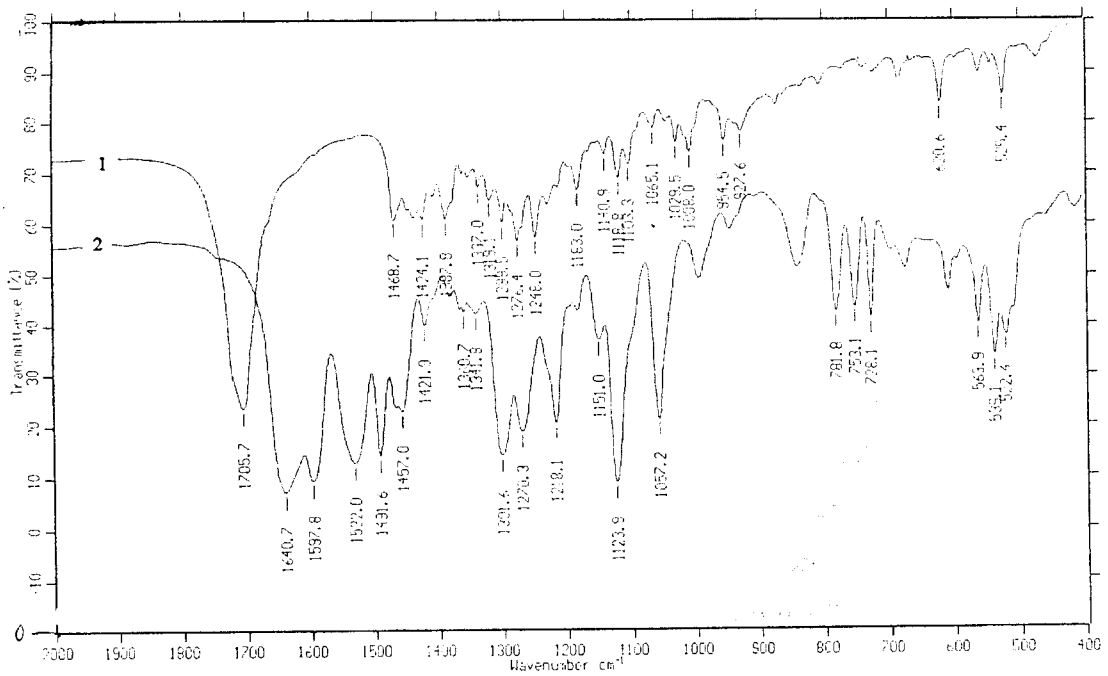

Figure 2. IR Spectrum of AMS and DHCA in KBr, 1- DHCA, 2- AMS

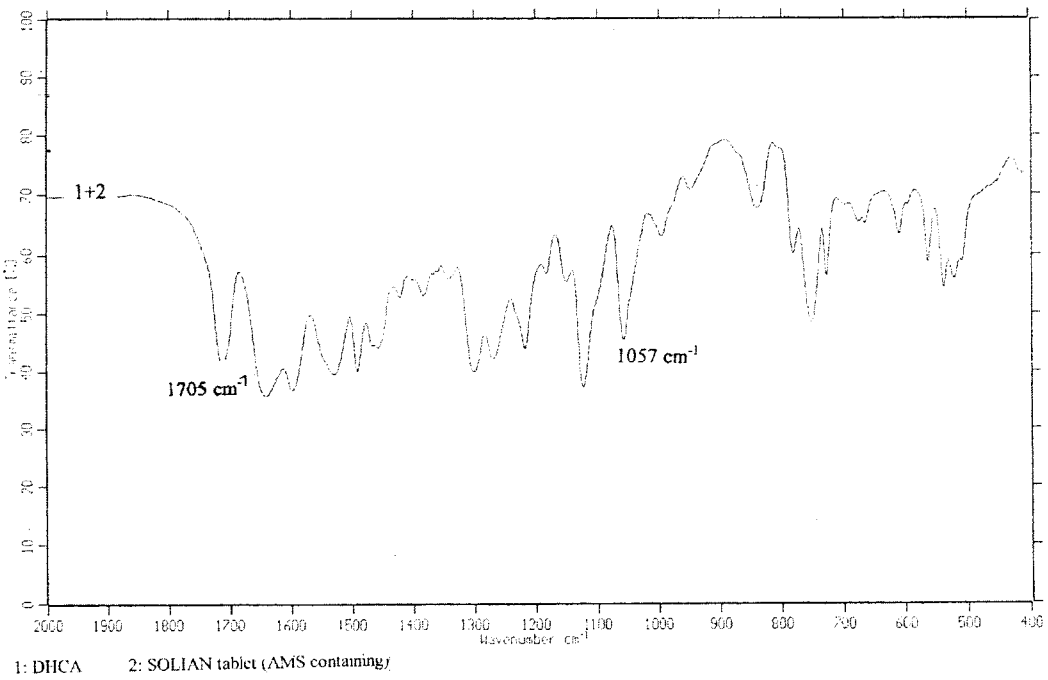

Figure 3. IR Spectrum of commercial tablet (SOLIANR) containing DHCA in KBr.

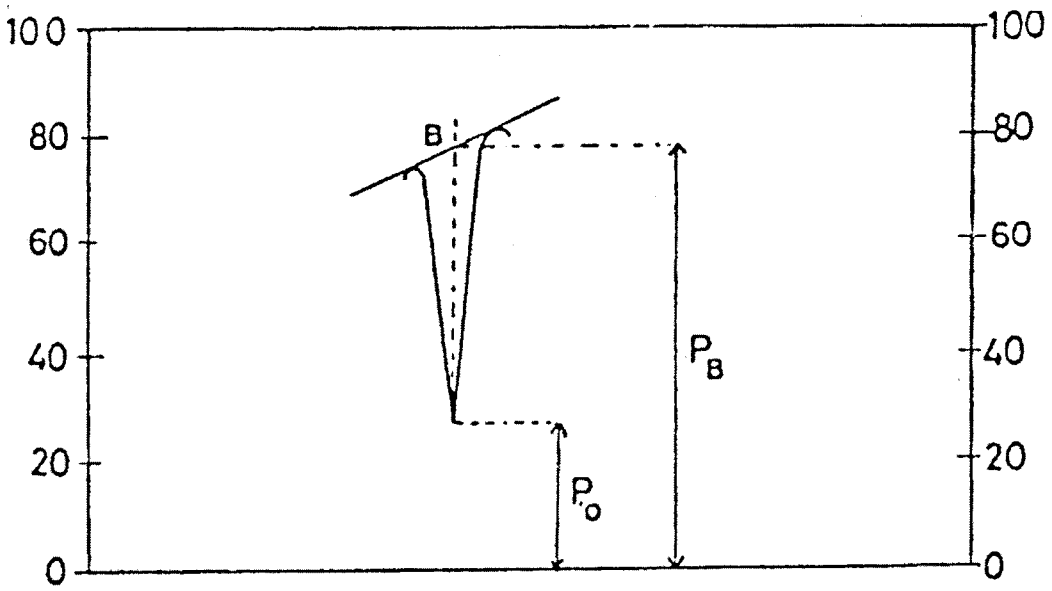

Figure 4.Application of base-line tecnique

TABLE 1 . $LogP_B$ -Log P_o values found for AMS-DHCA in synthetic standard mixtures.

Synthetic Standart Mixtures	Disc weight (mg)	AMS 1057 cm^{-1}			DHCA 1705 cm^{-1}				
		Conc. (mg)	P_B -P_o	$LogP_B$ -log P_o	Conc. (mg)	P_B –P_o	$logP_B$ -log P_o	$X=C_{AMS}/C_{DHCA}$	$y= T_{AMS}/T_{DHCA}$
St_1	125.2	0.988	71.0-55.0	0.1103	0.7410	65.2-37.0	0.246	1.33	0.4484
St_2	126.0	1.968	65.2-44.2	0.1689	0.9843	60.1-32.8	0.263	3.00	0.8818
St_3	124.0	2.412	60.4-40.0	0.1790	0.9650	60.0-34.0	0.247	2.00	0.6422
St_4	123.8	1.462	66.6-43.4	0.1575	0.4874	64.5-42.7	0.179	3.33	0.9759
St_5	123.5	2.407	60.9-41.9	0.1620	0.7220	61.6-42.0	0.166	2.50	0.7247

C_{AMS}= AMS conc. in KBr disc. T_{AMS}= $LogP_B$ -log P_o values of AMS

C_{DHCA}= DHCA conc. in KBr disc. T_{DHCA}= $LogP_B$ -log P_o values of DHCA

At 1057 cm^{-1} regression equation was found to be y= 0.2587 x + 0.1043 (r= 0.9974). The mean percentage recoveries were found to be 100 ± 2.24 % (Table 2 and 3).

with base-line technique (12, 15) (Figure 4). The regression equation was formed by using the AMS/DHCA concentration ratio as (x) values and the ratio of LogPB-LogPo of AMS and LogPB-LogPo of DHCA values as (y) (Table 2). Absorbance values were measured by logarithmic substraction of PB and Po points. The regression equation was calculated by using the ratio concentration/absorbance of AMS and DHCA (Table 1).

TABLE 2 . Characteristics for regression equations for different methods.

Methods	r	Regression Equations
IR	0.9974	y= 0.2587 x +0.1043
HPLC	0.9990	y= 11.8440 x+ 0.0305
UV	0.9988	y= 0.0570 x+ 0.0207

The second procedure in this study is the application of HPLC for determination of AMS. The aim of this work was also to develop a simple and stability indicating gradient HPLC assay for the analysis of AMS in pharmaceutical samples.

In order to realize the simultaneous elution of AMS and ATN peaks under gradient conditions of mobile phase composition was optimized. AMS and internal standard were eluted forming well shaped symmetrical single peaks well separated from the solvent front. The elution order were AMS (t_r =6.14) and for ATN (t_r = 4.41) at a flow- rate 0.7 min^{-1}. Optimum separation was realized by using methanol-0.032M phosphat buffer (70:30)v/v, pH= 7± 0.1. Detection was carried out using a UV detector at 275 nm. Linear relationship in the range of 0.64-2 $mcg.mL^{-1}$ was obtained as y=11.844x+0.0305 (r=0.9990) where y= peak height ratios of AMS to the ATN (Table 2) and x= concentration ratio of AMS to ATN. The mean percentage recoveries were found to be 100.5±1.79% (Table 3)

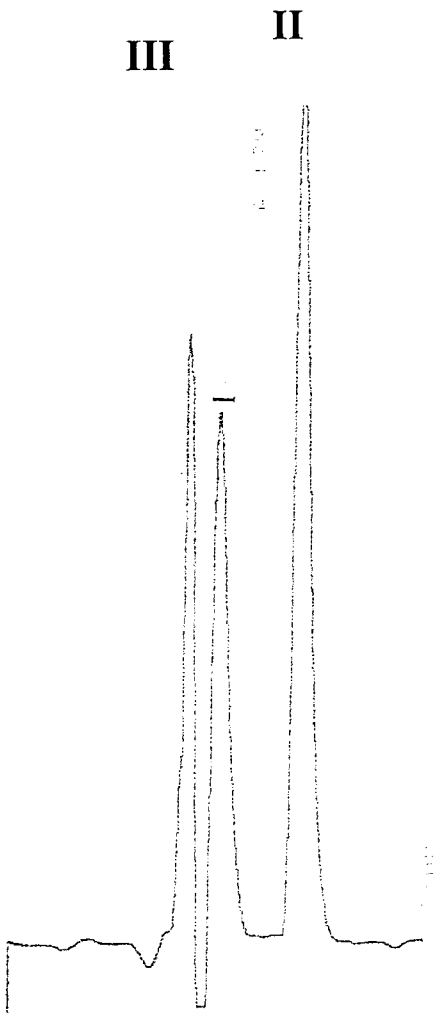

Figure 5. HPLC chromatogram of commercial tablet (Solian^R 200 mg tablet) solution in methanol-0.032 M phosphate buffer (70:30). **I**-ATN (Int. St. 10 mcg. mL^{-1} t_r= 4.41), **II**-AMS (1.6 mcg. mL^{-1} t_r= 6.14) , **III**-Solvent peak.

TABLE 3. Recovery results of synthetic standart mixtures by the proposed methods.

Synthetic Standard Mixture	IR Spectroscopy*			HPLC**			UV Spectroscopy**		
	mg			mcg. mL^{-1}			mcg. mL^{-1}		
	Added	Found	Recovery %	Added	Found	Recovery %	Added	Found	Recovery %
1	0.988	0.9753	98.7	0.64	0.638	99.8	4	4.02	100.5
2	1.968	2.015	102.40	0.96	0.967	100.7	6	6.11	101.8
3	2.412	2.352	97.50	1.28	1.293	101.2	8	7.94	99.2
4	1.462	1.489	101.9	1.60	1.592	99.5	10	10.22	102.2
5	2.407	2.440	101.3	1.92	1.964	102.3	12	12.07	100.6
	Mean		100.4			100.5			100.9
	RSD %		2.24			1.79			1.18

*Result obtained are the three determination for each mixture

** Result obtained are the five determination for each mixture

TABLE 4. Assay results of commercial samples (Solian^{R} tablet 200 mg AMS) with the proposed methods

Sample	Amount Found *(AMS)					
	IR		HPLC		UV	
	mg	%	mg	%	mg	%
1	204.4	102.2	203.6	101.8	198.4	99.2
2	197.4	98.7	200.8	100.4	201.5	100.7
3	195.0	97.5	199.0	99.5	197.4	98.7
4	193.5	96.7	197.6	98.8	198.2	99.1
5	195.1	97.5	205.4	102.7	203.2	101.6
Mean (x)	197.1	98.5	201.3	100.6	199.7	99.8
SD	4.95		3.2		2.47	
RSD %	2.49		1.54		1.24	
CL	x ± 6.2		x ± 3.9		x± 3.10	

SD: Standart Deviation, RSD: Relative Standart Deviation, CL: Confidence Limit

UV spectroscopy was used as an other comparison method for quantitative determination of AMS in commercial tablets. A linear concentration was obtained as 2-18 mcg.mL^{-1} in this method. The regression equation was found as y = 0.0507x+0.0207 and correlation coefficient is (r= 0.9988). $\in$ and A_1^1 (1%, 1cm^{-1}) were calculated as 19388 and 525 respectively in methanol. The mean percentage recoveries was found to be 100.9±1,18% (Table 2).

The result obtained for AMS determinations in commercial samples by using three methods (Table 3 and 4) were compared with student's t and Fischer F test statistically. These results showed that the differences between the result of the methods were statistically insignificant (Table 5).

TABLE 5. Statistically comparison of the results of proposed methods

Method	Student t test	Fischer F test
IR-HPLC	1.58	4.02
IR-UV	1.14	2.39
HPLC-UV	0.894	1.68

n(10-2= 8) p= 0.05

t theoritical value= 1.86

F theoretical value= 6.39

Conclusion

In literature no IR spectroscopic method for the AMS quantification has been reported. In this study application by IR spectroscopic method for the determination of AMS was proposed for the first time. Suggested method can be used as an alternative determination method in solid dosage forms containing AMS as the active compound. Also a simple and stability indicating gradient HPLC assay was developed for the analysis of AMS in pharmaceutical samples.

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