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Research Article

Quantitative Determination Of Sofosbuvir In Pure And Pharmaceutical Dosages Form By ATR-FTIR Spectroscopic As A Green Method

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ABSTRACT

Background:
To assess the concentration of sofosbuvir (CAS-1190307-88-0) in pharmaceutical formulations as well as in its pure form, a unique green analytical chemistry approach was developed. ATR-FTIR technique was used to quantitatively evaluate sofosbuvir, a multipurpose antiviral drug, in both tablet and bulk forms.
Objective:
Sofosbuvir in pharmaceutical goods may be quickly detected by the development and confirmation of an accurate, sensitive, and non-invasive attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy approach.
Methods:
Sofosbuvir is analysed using a method that looks for the distinctive skeletal band for C-O-C stretching in the 1170–1000 cm–1 spectral region. These procedures, which have linear ranges for SBR of 1.0 to 7.0 mg g-1, were fully verified in compliance with ICH recommendations.
Results:
The correlation value was 0.9993, and the linearity ranged from 1.0 to 7.0 milligrammes. The results indicated that the limits of quantification (LOQ) and detection (LOD) were 0.142 ± 0.09 mg and 0.079 ± 0.02 mg, respectively.
Conclusions:
Green chemistry guided the development of the analytical approach, and it was shown that the outcomes were exactly in line with destructive methods.
Highlights: It was shown that the results produced were in perfect accord with destructive methods, and the analytical strategy was designed in light of green chemistry.

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INTRODUCTION

The ageing population of persons infected with the hepatitis C virus (HCV) has caused a rise in the death rate from chronic HCV infection in several countries [1-3]. Over 0.5 million people die each year from problems related to HCV, including liver cancer, cirrhosis, and organ failure, out of the approximately 115 million people who have tested positive for the virus [1-3]. The advent of direct-acting anti-HCV drugs (DAAs) has brought about a substantial change in the treatment of chronic HCV infection, allowing for beneficial and efficient therapy in all instances. The FDA has approved Sofosbuvir (SBR), a second-generation DAA drug. It stops viral replication by functioning as an NS5B RNA-dependent RNA polymerase inhibitor, the nucleotide counterpart of the hepatitis C virus [4,5] Please see Figure 1. Oral administration of sofosbuvir, an anti-hepatitis C virus (HCV) drug, is possible, and it has a wide genetic coverage [5]. Sofosbuvir undergoes metabolic mechanisms in the liver that result in the sequential hydrolysis, phosphoramidate cleavage, and phosphorylation of its active triphosphate nucleoside analogue [6]. These directly acting antiviral drugs were quantified using a variety of approaches, such as spectroscopic and chromatographic methods. Organic solvents are frequently used extensively in traditional analytical methods for analysing these drugs, whether they are in medicinal dose forms or bulk [6–9]. The goal of green analytical chemistry (GAC) is to use analytical techniques and approaches that reduce or completely eradicate the use of compounds that might be hazardous to the environment or public health [10]. Green chemistry concepts must be incorporated into analytical technique design in order to minimise the negative impacts of chemicals and equipment on the environment and human health [10,11]. Investigating novel direct techniques that do not use organic solvents or reagents is one way to reduce the environmental impact of analytical procedures [10]. Rapid analytical methods such as Fourier Transform Infrared (FTIR) spectroscopy provide a wealth of qualitative and quantitative information from solid materials [12]. Therefore, infrared spectroscopy offers a substantial benefit when used directly for descriptive and analytical purposes of active pharmaceutical ingredients (APIs) in their solid-state (liquid-free analytical techniques) within the context of green analytical chemistry (GAC). In addition, the ATR-FTIR approach has many advantages over other spectrometric and chromatographic techniques. Because it doesn't affect solid sample materials, it allows active pharmaceutical ingredients (APIs) analytical times to be shortened without the influence of excipients. It does so by doing away with the necessity for chemical reagents in analytical procedures in an ecologically responsible manner [12,13]. In order to directly quantify SBR (Figure 1) in both its pure form and pharmaceutical formulations, the goal of this work is to develop a non-destructive, quick, accurate, economical, and environmentally friendly ATR-FTIR spectroscopic approach.

Experimental

Chemicals and materials

Sofosbuvir (assigned 99.6% w/w purity) was kindly provided as a gift sample by Mylan Laboratories Pvt. Ltd (Nashik, India). The potassium bromide was obtained from an Indian research facility and dried in an oven before usage to make sure that no water vapour remained. To be analysed, two commercially accessible tablets were bought from the local market: the 400 mg SoviHep pill from Zydus Healthcare Pvt. Ltd. (India) and the 400 mg Mylan Labs Pvt. Ltd. (India). The method's development used only analytical reagent (AR) grade chemicals for all other substances.

Instrumentation

Every FTIR measurement was conducted using a Bruker ALPHA-IIE FTIR Spectrometer, and Thermo Electron Corporation's (Madison, WI) OPUS-8.0 spectra software was used for data processing. The A210/D-11 ATR attachment, which has W303/D-U ZNSE Optics ideal for operation in high humidity settings, was used to handle several sorts of samples, including solids, liquids, powders, pastes, and viscous materials. An Atlas 15T hydraulic press (made by Specac Ltd.) was used to manually make potassium bromide discs for SBR analysis. Operating in a single reflection arrangement, the ATR sampling device used a diamond internal reflection device within a ZnSe support/focusing component. A Shimadzu AUY 220 electronic analytical balance (Japan) was continuously employed over the duration of the study. The experimental setting also included the use of FalconTM 50 mL Conical Tubes and porcelain mortar and pestle sets.

Analytical procedure

Individual weights of actual SBR powders falling within predetermined limits were transferred into 50 mL FalconTM conical tubes. A vortex mixer was used to fully mix the 1:1 ratio of crystalline potassium bromide powder that was introduced to each tube. The homogenised samples were vortexed to thoroughly mix them and decrease their shape after each addition of potassium bromide. FT-IR spectroscopy was used to analyse the resultant blends, capturing spectra in the mid-IR band (4000–400 cm-1) (Table 1) with a sensitivity of 2 cm-1. Every run was carried out using potassium bromide under the same ideal circumstances. The appropriate regression equation was obtained by graphing absorbance against the concentration of the final dosage to create calibration curves.

Assay of Sofosbuvir drug in pharmaceutical preparations

The suggested method differs from other techniques for measuring the studied SBR in pharmaceutical formulations since it just calls for the tablet dosage form to be ground into a powder in a porcelain mortar in order to lower the particle size before FTIR analysis. The general suggested methods described in the analytical technique section were followed with regard to the use of five replicate samples. Regression analysis was used to calculate the amounts of SBR medicines analysed. Also an analysis of SBR testing for content uniformity of tablet dosage form of two marketed formulation using the proposed FTIR method (Table 6).

RESULTS AND DISCUSSION

Direct API solid-state analysis decreases the harmful effect on the environment and public health while also being better suitable for the analysis of pharmaceuticals with solubility issues. As a tool for quantitative evaluation the calibre of analytical techniques employed throughout the pharmaceutical production process, FTIR had widely utilised to detect and quantify API for a range of pharmaceuticals in recent years [13-18, 40-65]. The qualitative study for the analysed SBR medications has been conducted by evaluating the standard references of the researched pharmaceuticals to assure the lack of impurities, breakdown products, or interference from naturally occurring compounds. The quantitative study was done by measuring the absorbance of skeletal band corresponding to C-O-C stretching within a spectral range of 1170–1000 cm-1 for the SBR medications that were tested (Figure 2). A chosen location for the unique skeletal band corresponding to aromatic ring confirms that no additives from typical pharmaceutical excipients interfered with the measurement of the examined SBR medicines in commercially sold tablet dosage form. Different concentration levels of the SBR medicines were used to create analytical FTIR spectra (Figure 4).

Method validation

Through ICH guidelines, the provided methods had wholly verified [19].

Range and linearity

The calibration curves had made by graphing the absorbance readings against their respective solutions at five different concentration levels. The correlation coefficient (r) of 0.9993 and the determination coefficient (rs) of 0.999 for SBR were achieved by aggregating three analytical calibration graphs of the drug. The statistical parameters for the mentioned drugs were calculated using the resulting linear regression analysis for the recommended approach, as Table 2 shows.

Quantitation and of detection limits

Using the formula LOQ = 10 S/b, where S is the mean of the standard deviation of the intercepts and b is the slope of the calibration graphs, the LOQ values for the medications under investigation were determined following ICH recommendations. LOD value was determined using the formula LOD = 3.3 S/b. The results showed that the LOQ and LOD were 0.142 mg/g and 0.097 mg/g, respectively.

Accuracy

Recovery experiments as a proposed ATR-FTIR approach had been carried out using SBR's regression equations, and each was examined at three distinct concentration levels (80, 100, and 120 percent). The acceptable accuracy of the recommended approach has been established, as shown in Table 3, by comparing the % recovery resulting values to the actual values.

Precision

Three concentration levels within the prescribed linear range of SBR genuine powders were evaluated for three days to determine inter-day precision (intermediate precision). Intra-day accuracy (repeatability) has been dedicated by measuring three concentration levels of SBR genuine powders within the prescribed linear range on the same day. Table 4 revealed that the recommended approach had good precision at the intermediate precision and repeatability precision stages, as the % RSD value for intra-day precision was 1.28, and the % RSD value for inter-day precision was 1.11 for SBR.

APPLICATIONS

Application to marketed injection

Sofosbuvir in its pharmaceutical injectable form were analysed using the present analytical technique (MYHEP 400 mg and SOVIHEP 400 mg tablet). The proposed ATR FTIR method's label claim % (recovery ± SD) was 100 ± 1.1 for SBR, respectively. The Student's t-test and the variance ratio F-test has been utilized to evaluate the findings of the recommended and accessible spectroscopic methods [9,18]. As the calculated values seem to be lower than the corresponding values, illustrating the same precision and accuracy within the evaluation of SBR drug in its tablet dosage through proposed technique, there has been no appreciable difference among the results obtained by calculation of the recommended ATR-FTIR system and results acquired by the destructive spectroscopic methods (Table 5) [22-65].

CONCLUSION

This present study provides a fast and reliable ATR-FTIR technique for analysing remdesivir in both API material and pharmaceutical dose forms. The suggested method has the benefit of allowing for direct API drug of the investigated without the necessity for pretreatment of a sample in solid form, and also the elimination of organic chemical solvents from the analytical process. In order to make sure the SBR drugs under analysis were devoid of contaminants, degradation byproducts, or interference from natural substances, a qualitative study of the drugs entailed evaluating their standard references. The skeletal band of C-O-C stretching absorbance for the evaluated SBR medicines was measured for the quantitative analysis, with measurements taken within the

spectral region of 1170–1000 cm–1. We confirmed the absence of common pharmaceutical additive interference in the commercially available tablet dosage form of the SBR drugs under investigation by carefully choosing particular positions for the unique carbonyl bands. The LOD was 0.079 ± 0.02 mg/g, and the LOQ was 0.142 ± 0.09 mg/g respectively. The percentage RSD values for intra-day precision and inter-day precision for SBR were 1.28 and 1.11, respectively, indicating the intermediate precision and repeatable precision phases. The label claim percentage (recovery ± SD) for SBR using the suggested ATR FTIR technique was 100 ± 1.1. The advantages of the proposed approach include the ability to directly use the researched API medication without requiring the preparation of a solid sample and the removal of organic chemical solvents from the analytical procedure.

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The authors do not have any conflict of interest

AUTHORS' CONTRIBUTIONS:

All authors have read and approved the manuscript. SR contributed in preparation primary content. He performed extensive literature survey and compile the content. SR contributed in preparation of figures and table. SB contributed in checking of manuscript and correction of grammatical mistake. SR contributed in preparation of figure. SB contributed in finalization of manuscript and in its correction. SB contributed in finalization of content, preparation of concrete manuscript and in schematic presentation of content.

ABBREVIATIONS

HCV	Hepatitis C virus
SBR	Sofosbuvir
DAA	Direct-acting anti-HCV
NS5B	Nucleotide analogue
GAC	Green analytical chemistry
FDA	Food and drug administration
API	Active pharmaceutical ingredients
HPLC	High-performance liquid-chromatography
HPTLC	High-performance thin-layer chromatography
LC-MS	Liquid-chromatography-mass spectroscopy
ATR	Attenuated total reflection
FTIR	Fourier transform infrared spectroscopy
LOD	Limit of detection
LOQ	Limit of quantification
RSD	Relative standard deviation
SD	Standard deviation
ICH	International council for harmonisation
IV	Intravenous

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Figures:

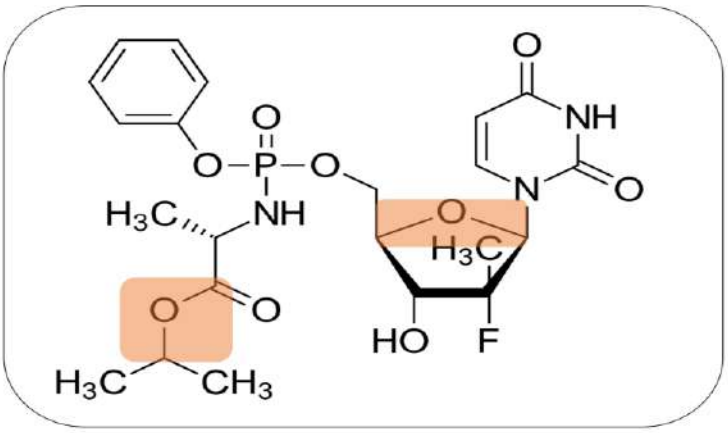

Figure 1: Structure of Sofosbuvir

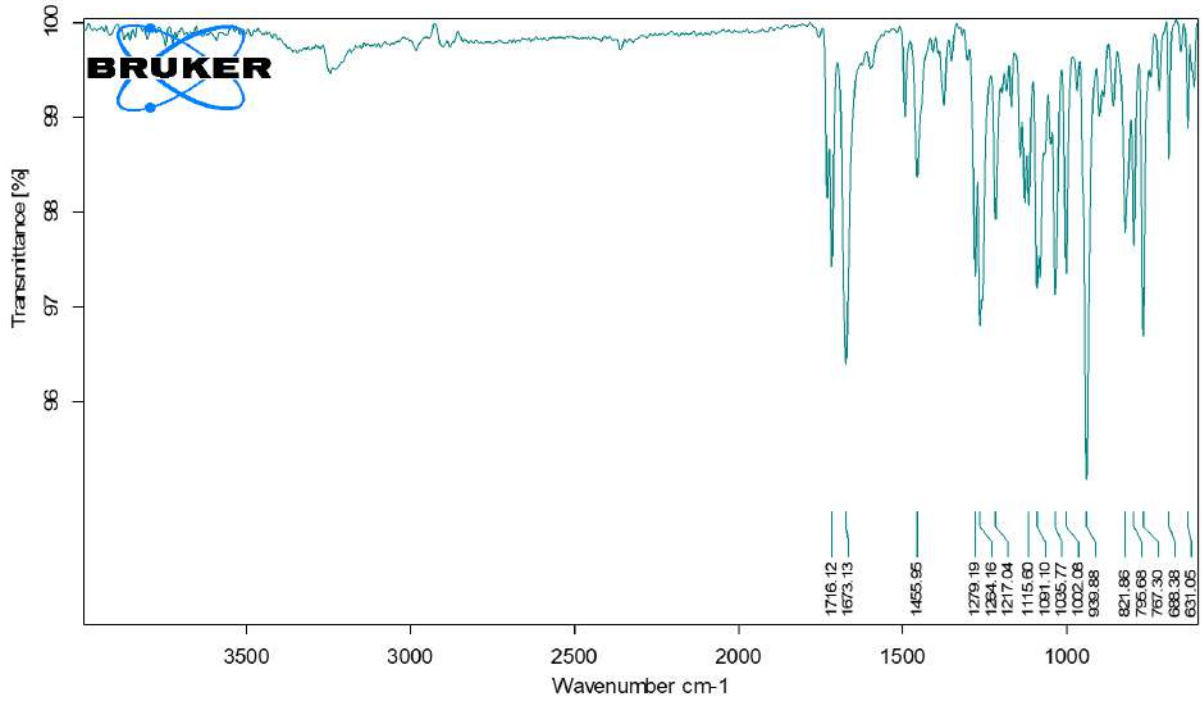

Figure 2: Transmittance IR spectra of pure sample (API).

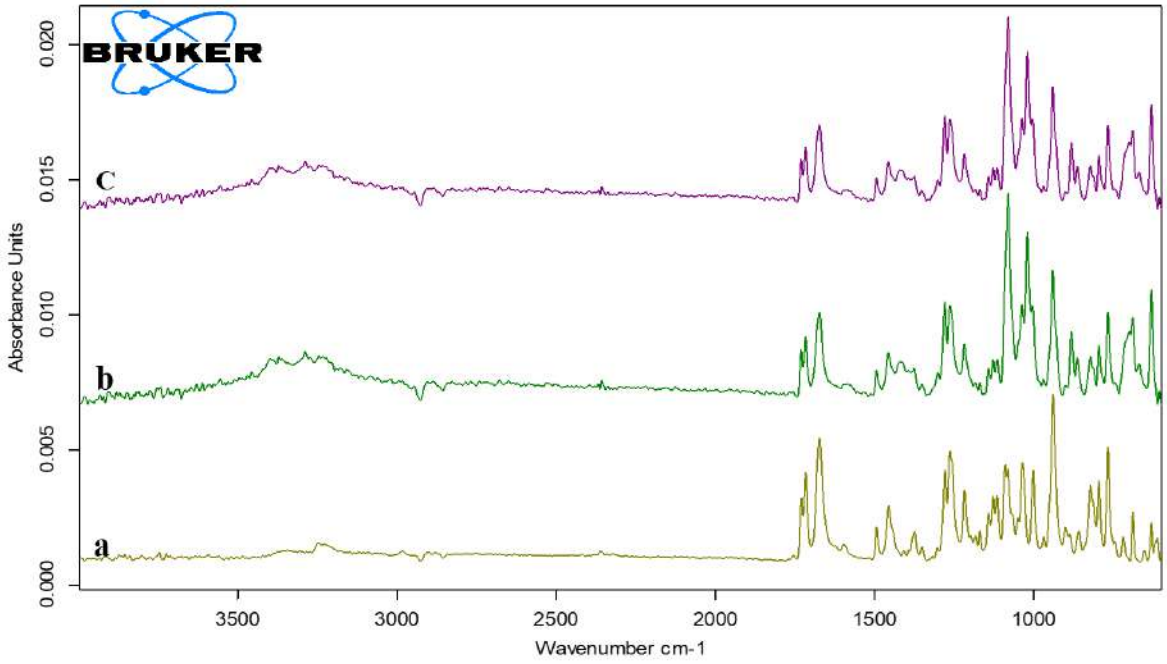

Figure 3: Overlap of IR spectra referring to the (a) 150 mg pellets of sample (API), (b) reference sample (marketed sample-1), and (c) reference sample (marketed sample-2).

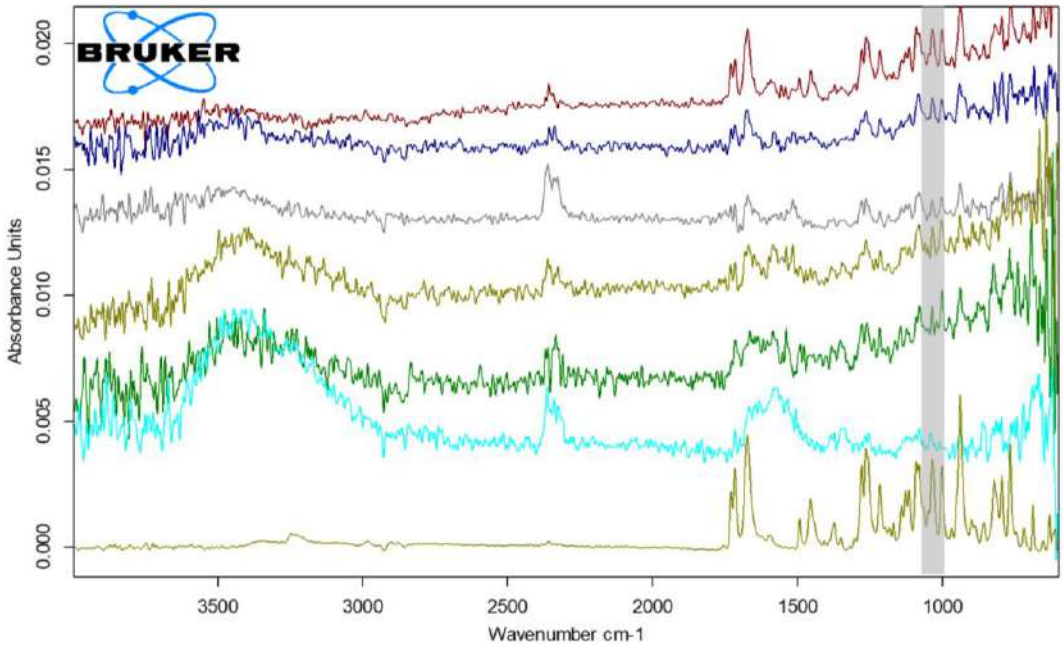

Figure 4: Skeletal band for aromatic ring (C-H Band) of Sofosbuvir at various concentration

.

Tables:

Table 1 IR spectral analysis of Sofosbuvir

Frequency	Functional group
3331	N–H stretch
3525	O–H stretch
2935, 2897	C–H stretch
1729	C=O stretch
1617, 1582, 1414	C=C ring stretch
1400-1600	Aromatic ring (C-H band)
1170–1000	C–O–C stretch
805, 688	C=H bend, C=C ring bend

Table 2 Linearity and range study

Sr. No.	Parameter	Results
1.	Range (mg/g)	1.0 -7.0
2.	Slope	2.24 X10-2
3.	SD of slope (Sb)	2.7 X 10-4
4.	Intercept	4.9 X 10-2
5.	SD of Intercept (Sa)	4.1 X 10-3
6.	Correlation coefficient (r)	0.9993
7.	Determination of coefficient (r2)	0.9999
8.	Number of determinations	7
9.	Limit of Determination (mg/g)	0.079 ± 0.02
10.	Limit of Quantification (mg/g)	0.142 ± 0.09

Table 3: Evaluation of accuracy for the analysis of SBR.

Drug	Taken (Mg/g)	Added Concentration (%)	Recovered amount (%)	% RSD
MYHEP 400mg	5	80 (2 mg)	100.23 ± 0.21	0.21
	5	100 (5 mg)	99.89 ± 0.14	0.14
	5	120 (7 mg)	100.74 ± 0.23	0.23
SOVIHEP 400mg	5	80 (2 mg)	99.78 ± 0.31	0.31
	5	100 (5 mg)	101.31 ± 0.19	0.19
	5	150 (7 mg)	100.69 ± 0.24	0.24

***Value presented as mean value of three determination (n=3)**

Table 4: Precision data for the analysis of SBR by proposed FTIR method.

Sr. No.	Interday precision (intermediate precision)			Intraday precision (repeatability precision)		
	Conc. Level	Average peak area	% RSD	Conc. Level	Average peak area	% RSD
1	5	735.17	0.98	5	724.56	0.88
2	10	1389.11	0.76	10	1383.81	0.55
3	15	2202.16	1.21	15	2201.18	1.72
		Mean % RSD	0.98		Mean % RSD	1.05

***Value presented as mean value of three determination (n=3)**

Table 5: Determination of SBR in formulation.

Commercial product	SBR (10 mg)	
	MYHEP 400mg	SOVIHEP 400mg
Amount found (mg)	9.953	9.987
% Purity ± SD	99.79 ± 1.72	99.84 ± 1.91
t-value	1.385	1.389
F-value	2.468	2.486

***Value presented as mean value of three determination (n=3)**

Table 6: Analysis of SBR testing for content uniformity of dosage units using the proposed FTIR method.

Parameter	Weight of tablet (gm)	Content Uniformity
	1.2412	99.83
	1.2658	101.07
	1.2656	102.42
	1.2427	98.91
	1.2520	100.63
	1.2501	100.63
	1.2649	101.07
	1.2548	99.39
	1.2679	101.64
	1.2480	99.87
	1.2495	103.31
	1.2485	99.98
	1.2428	100.12
	1.2337	101.02
	1.2473	99.37
Mean	1.2516	100.61
SD	0.0099	1.15
AV	-------	5.01