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REVIEW ARTICLE

Forced Degradation and Stability Indicating Chromatographic Methods for the Analysis of Sofosbuvir Alone and in Combination with Velpatasvir, Daclatasvir, Voxilaprevir and Ledipasvir

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SUMMARY

Sofosbuvir (SOF) is an antiviral compound used alone to treat hepatitis C or in combination with drugs such as ribavirin and ledipasvir (LED). FDA approval as monotherapy was granted in 2013, and for combination treatment of hepatitis C in 2014. Different studies have reported on the analysis of SOF in bulk and tablet forms. However, a monograph for SOF has not yet been included in official pharmacopeias. Therefore, no consensus with respect to the identification of impurities and concerns relating to the safety of the drug exists. A review of the development of stability indicating chromatographic methods for the analysis of SOF was undertaken using PubMed and the Google Scholar databases from initial reports to January 2023. Our focus pertained to studies in which a stability-indicating chromatographic method had been designed and validated for analysis of SOF in bulk and tablet form alone and in combination with LED, daclatasvir (DAC), velpatasvir (VEL) and voxilaprevir (VOX) and also reported the use of stress testing. This review aims to summarize the information reported in different studies concerning the development of stability-indicating methods conducted using stress studies to analyze SOF and the results of such stress studies.

Key Words: *Sofosbuvir, anti-virus drug, stability indicating, method development, stress studies.*

Sofosbuvir'in Tek Başına ve Velpatasvir, Daklatasvir, Voksilaprevir ve Ledipasvir ile Kombinasyonunun Analizi İçin Hızlandırılmış Degradasyon ve Stabilite Göstergesi Kromatografik Yöntemler

ÖZ

Sofosbuvir (SOF), hepatit C tedavisinde tek başına ya da ribavirin ve ledipasvir (LED) gibi ilaçlarla birlikte kullanılan antiviral bir bileşiktir. Monoterapi olarak FDA onayı 2013 yılında, hepatit C'nin kombinasyon tedavisi için ise 2014 yılında verilmiştir. Bulk ve tablet formlarında SOF analizine ilişkin farklı çalışmalar rapor edilmiştir. Ancak SOF için bir monografi henüz resmi farmakopelerde yer almamıştır. Bu nedenle, safsızlıkların tanımlanması konusunda bir fikir birliği oluşmamış olup, ilacın güvenliğine ilişkin endişeler de bulunmaktadır. SOF analizi için stabiliteyi gösteren kromatografik yöntemlerin geliştirilmesine ilişkin bir inceleme, ilk raporlardan Ocak 2023'e kadar PubMed ve Google Akademik veri tabanları kullanılarak gerçekleştirildi. Odak noktamız, SOF'un bulk ve tablet formunda tek başına ve LED, daklatasvir (DAC), velpatasvir (VEL) ve voksilaprevir (VOX) ile kombinasyonu halinde analizi için stabiliteyi gösteren bir kromatografik yöntemin tasarlandığı ve doğrulandığı ve ayrıca stres testinin kullanımını bildiren çalışmalara yönelikti. Bu derlemenin amacı, SOF'u analiz etmek için stres çalışmaları kullanılarak yürütülen stabilite gösterge yöntemlerinin geliştirilmesine ilişkin farklı çalışmalarda bildirilen bilgileri ve bu stres çalışmalarının sonuçlarını özetlemektir.

Anahtar Kelimeler: *Sofosbuvir, antiviral ilaç, stabilite göstergesi, yöntem geliştirme, stres çalışmaları.*

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INTRODUCTION

Sofosbuvir (SOF) is a nucleotide analog NS5B polymerase inhibitor used to treat chronic hepatitis C infection caused by genotypes 1, 2, 3, or 4 in adult patients. (Singh, Bhatt, & Prasad, 2017). It is a direct-acting antiviral compound used alone or in combination with other drugs such as ribavirin and ledipasvir (LED) (Wang et al., 2017; Nebsen et al., 2016) and was approved in 2013 for monotherapy and in 2014 for combination treatment of hepatitis C (WHO, 2016). This compound is better than previously approved therapies as it exhibits better recovery rates and fewer side effects achieved with shorter duration treatment times (Wang & You, 2017). Currently, SOF is produced in many countries globally and different studies relating to analysis in bulk and tablet forms have been undertaken (Agarwal et al., 2022; Ganji et al., 2021; Hamdache et al., 2021; Abdel-Razeq et al., 2019; Bhujbal et al., 2019; Annapurna et al., 2018; Hassouna et al., 2018; Lalitha et al., 2018; Vanitha et al., 2018; Shaikh et al., 2017; Swathi et al., 2017; Nebsen et al., 2016; Pottabathini et al., 2016; Swain et al., 2016; Vejendla et al., 2016). In addition, a study using a Quality by Design (QbD) approach has been used for the development and validation of analytical methods for this drug (Bhujbal & Darkunde, 2019) has been reported and others have estimated SOF content in pharmaceutical formulations (Shaikh & Manjusri, 2017; Vejendla, Subramanyam, & Veerabhadram, 2016). Several stability indicating methods have been designed to simultaneously estimate SOF with LED, daclatasvir (DAC), velpatasvir (VEL) and voxilaprevir (VOX) (Balaswami, Ramana, Rao, & Sanjeeva, 2018; Bandla & Ganapaty, 2017; Bandla & Ganapaty, 2018; Bhavani & Maduri, 2020; Damle & Kalaskar, 2020; Deepthi & Sankar, 2020; El-Waey, Abdel-Salam, Hadad, & El-Gindy, 2023; El-Yazbi, Elashkar, Abdel-Hay, Talaat, & Ahmed, 2020; R Godela & Sowjanya, 2020; Ramreddy Godela & Sowjanya, 2021; Harshalatha, Chandrasekhar, & Mv, 2018; Hassouna, Abdelrahman, & Mohamed, 2017; Hemchand, Babu, & Annapurna, 2018; Jahnavi & Ganapaty, 2018; Kokkirala & Suryakala, 2020; Kumar & Rao, 2018; Kumari & Sankar, 2019; Lakshmana Rao & Pallavi, 2019; Lakshmi, Chaitanya, & Chandrasekar, 2018; Lakshmi Maneka S, Saravanakumar RT, & Anjana, 2020; Mankar, Bhawar, & Dalavi, 2019; Mastanamma, Chandini, Reehana, & Saidulu, 2018; Namratha & Vijayalakshmi, 2021; Narla & Pappula, 2020; Padmini M, Venkata D, & Sankar, 2019; Priyanka, Vinutha, Sridevi, Ramya, & Bhagavan Raju, 2018; Rao, Reddy, & Rao, 2017; Rao, Rao, & Prasad, 2018; Reddy, Alam, Khanam, & Adhakrishnanand, 2018; Rote, Alhat, & Kulkarni, 2017; Saroja, Lakshmi, Rammohan, Divya, & Kumar, 2018; Suganthi, Satheshkumar, & Ravi, 2019; Susmita & Rajitha, 2018; Veereswara Rao, Deshmukh, & Kumar, 2018; Yeram, Hamrapurkar, & Mukhedkar, 2019; Zaman & Hassan, 2021).

The presence of impurities and degradation products (DPs) may affect the efficacy and safety of drugs. Consequently, it is essential to conduct stability studies to identify such impurities (Fakhari, Nojavan, Haghgoo, & Mohammadi, 2008). Drug stability is an important quality attribute for the pharmaceutical industry, and analytical methods designed for quality control should preferably be stability-indicating (Montazeri, Mohammadi, Adib, & Naeemy, 2018). According to the International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use and USP guidelines, the assay method for a drug-active substance must be stability-indicating to permit determination of the active substance in the presence of any potential DPs of that compound (Souri et al., 2011). The best approach for developing stability-indicating analytical methods is to undertake forced degradation studies during method development and validation (Pourmoslemi, Mirfakhraee, Yaripour, & Mohammadi, 2016). There are several analytical techniques used for the analysis of drugs, of which HPLC methods are commonly used for quality control and consistency of medicines as the approach is reliable, simple, cost-effective, and robust, and the familiarity of the analysts with this technique is a reason for the plethora of separations reported using this approach (Mohammadi et al., 2007). Therefore, our focus in this review is on the

chromatographic methods that have reported the analysis of SOF only or in combination with other antivirus drugs and the purpose of the review is to summarize information relating to the conduct of stress studies, results of such stress studies in reports in which the design and validation of stability indicating chromatographic methods of analysis for SOF had been published. For this purpose, we report information from articles that have reported the design of stability indicating chromatographic methods for SOF alone or with other antiviral drugs. For this purpose, the inclusion and exclusion criteria for this review resulted in 45 studies being considered suitable for inclusion from the scientific databases searched.

METHODS

Literature search

A review of the design, development, and validation of stability-indicating methods for the analysis of SOF was performed up to the beginning of 2023 using the updated PubMed and Google Scholar databases. In addition, the references from the list of identified and subsequently selected articles were reviewed to identify additional sources. Our focus was on chromatographic studies that involved the development and validation of stability-indicating methods of analysis for SOF alone or with other antivirus drugs simultaneously, during which stress testing was performed. Studies in which SOF was analyzed simultaneously with other drugs were considered in the inclusion criteria, and only chromatographic studies that analyzed the drug were included in our review. Only studies published in English were included. All selected reports were saved in an Endnote library, after which duplications were removed, and the titles and abstracts were screened to establish whether they met the inclusion criteria.

Search strategy

Our search strategy for Google Scholar and PubMed databases is depicted in (Figure 1.).

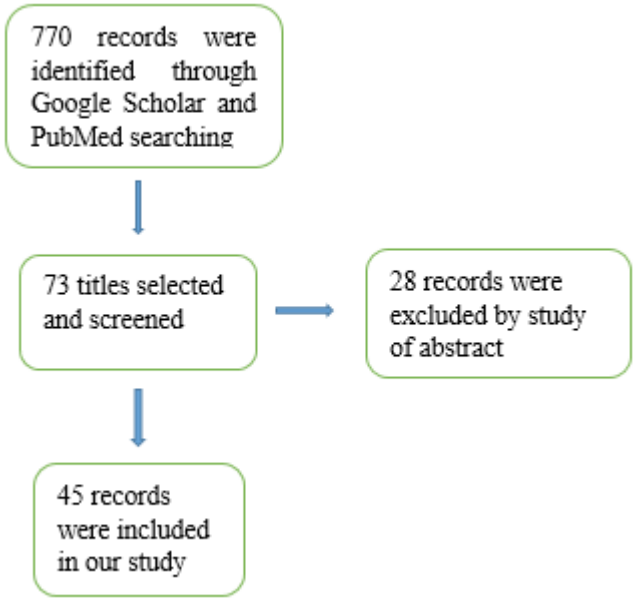

Figure 1. Flow Diagram of Study Selection Process

FINDINGS AND DISCUSSION

Summary of studies conducted with SOF alone

Ten studies were finally identified, selected and used following review of the actual analytical approach for SOF alone (Abdel-Razeq, Nasr, & S Said, 2019; Agarwal, Jagdale, & Gandhi, 2022; Annapurna, Teja, & Chaitanya, 2018; Hamdache et al., 2021; Hassouna & Mohamed, 2018; Lalitha, Reddy, & Devanna, 2018; Nebsen & Elzanfaly, 2016; Pottabathini, Gugulothu, Kaliyaperumal, & Battu, 2016; Swain et al., 2016; Vanitha, Bhaskar Reddy, & Satyanarayana, 2018).

The study conducted by Nebzen et al. aimed to investigate the degradation behavior of SOF under different stress conditions through a green validated stability-indicating method. The optimized LC-MS-MS method was used to identify fragmentation patterns of DP of SOF (Nebsen & Elzanfaly, 2016). The primary purposes of a study conducted by Pottabathini were to develop a stability-indicating method for the analysis of SOF, investigate the degradation behavior of the drug, separate and characterize the DP (Pottabathini, Gugulothu, Kaliyaperumal, & Battu, 2016). In another study, structural information generated using MS measurements was used for in silico toxicity studies of the DP, which were performed using two toxicity prediction software packages. The primary peak was well separated from the peaks of the DP, which were also resolved from each other, confirming the method was selective and stability-indicating. The LC-MS method used a LC-ESI-QTOF-MS/MS to identify the DP (Swain et al., 2016). In a study by Vanitha et al., a QbD approach was used to develop a simple, robust and selective RP-HPLC method for the estimation of SOF active pharmaceutical ingredient (API) in which degradation studies were performed under different stress conditions for the purposes of method optimization (Vanitha, Bhaskar Reddy, & Satyanarayana, 2018). Annapurna et al. designed a stability-indicating RP-UFLC method for the analysis of SOF in bulk form. For this purpose, forced degradation studies were performed under acidic, alkaline, oxidation, thermal and photolysis conditions. The specific and selective method was validated using ICH guidelines (ICH, 1997) and was applied to the analysis of SOF in commercial formulations (Annapurna, Teja, & Chaitanya, 2018). In a study conducted in 2022, a stability-indicating RP-HPLC method for drug analysis was reported and included forced degradation studies performed according to ICH guidelines (Guideline, 2003). Samples were analyzed by MS (Mass Spectrometry), and drug degradation pathways were identified and profiled (Agarwal, Jagdale, & Gandhi, 2022). Two stability-indicating methods using UPLC and HPTLC were developed and validated according to ICH guidelines for the determination of SOF (bulk form) in the presence of its DPs and included performing stress tests under acidic and alkaline hydrolytic and oxidative conditions (Abdel-Razeq, Nasr, & S Said, 2019). In three other studies, a stability-indicating RP-HPLC method was developed and validated to determine SOF and included degradation studies carried out under acidic, alkaline, oxidation, thermal, and photolysis conditions (Hamdache et al., 2021; Hassouna & Mohamed, 2018; Lalitha, Reddy, & Devanna, 2018).

A summary of the stress conditions and the extent of SOF degradation under different stress conditions used in each study is listed in (Table 1). In Study No. 3, stress tests were performed on the tablet form, and in Study No. 10, stress tests were performed on both bulk and tablet dosage forms.

Table 1. Summary of stress studies and % degradation of SOF

Study (Form) and %Degradation	Hydrolysis			Oxidation	Thermal	Photolytic	Reference
	Acidic	Basic	Neutral				
Study 1 (Bulk)	0.1 N HCl 60°C 30 min	0.1 N NaOH 25°C 2 min	NR[1]	30% v/v H_2O_2 60°C 1 h	60°C 1 h (In solution state)	UV 48 h (In solid state)	(Annapurna et al., 2018)
%Degradation	11.65%	27.03%	NR	32.85%	ND[2]	ND	
Study 2 (Bulk)	0.1 M HCl 25°C 6 h	0.1 M NaOH 25°C 6 h	Water 72 h	3%, 6% v/v H_2O_2 25°C 10 days	80°C 72 h In oven (In solid state)	UV 6 h 254 nm (In solid state)	(Nebsen & Elzanfaly, 2016)
%Degradation	2.5%	90%	ND	11%	ND	ND	
Study 3 (Tablet)	1 N HCl 80°C 10 h	0.5 N NaOH 60°C 24 h	NR	30% v/v H_2O_2 80°C 2 days	Thermal study was performed but NR	254 nm 24 h (In solid state)	(Pottabathini et al., 2016)
%Degradation	8.66%	45.97%	NR	0.79%	ND	ND	
Study 4 (Bulk)	0.1 M HCl 60°C 3 h	0.1 M NaOH 25°C 15 min	(Water: Acetonitrile 50:50% v/v) 60°C 6 h	30% v/v H_2O_2 25°C 72 h	80°C In oven 48 h (In solid state)	UV (200 $Whrm^{-2}$) Fluorescent light (1.2 million lux hours) (In solid state)	(Swain et al., 2016)
%Degradation	Two DPs	Three DPs	One DP	One DP	ND	ND	
Study 5 (Bulk)	0.1 M HCl 25°C 25 h	0.1 M NaOH 25°C 100 min	Water 25°C 72 h	0.3% v/v H_2O_2 25°C 50 h	60°C In oven 72 h (In solid state)	Sunlight 10 h (In solid state)	(Vanitha et al., 2018)
%Degradation	One DP	Two DPs	NR	ND	ND	NR	
Study 6 (Bulk)	0.1N HCl 70°C 6 h	0.1N NaOH 70°C 10 h	NR	3% v/v H_2O_2 25°C 7 days	50°C 21 Days (In solution state)	Sunlight 21 Days (In solution state)	(Agarwal et al., 2022)
%Degradation	One DP	One DP	NR	One DP	ND	ND	
Study 7 (Bulk)	1N HCl 50-60°C 14 h	1N NaOH 50-60°C 14 h	Water 50-60°C 48 h	3% v/v H_2O_2 25°C 48 h	75% Humidity 40°C 1 Month (In solid state)	Sunlight 48 h (In solid state)	(Abdel-Razeq et al., 2019)
%Degradation	18.36%	70.81%	NR	4.56%	4.31%	7.03%	
Study 8 (Bulk)	5N HCl 100°C 7 h	5N NaOH 100°C 3 h	NR	3% v/v H_2O_2 25°C One week	NR	NR	(Hassouna & Mohamed, 2018)
%Degradation	25%	19%	NR	32%	NR	NR	
Study 9 (Bulk)	0.1N HCl 60°C 6 h	0.1N NaOH 60°C 6 h	NR	3% v/v H_2O_2 25°C 15 min	110°C 24 h (In solid state)	Sunlight 24 h (In solution state)	(Lalitha et al., 2018)
%Degradation	6.7%	8.9%	NR	8.1%	5.1%	8.5%	
Study 10 (Bulk And Tablet)	1N HCl 8°C 3 h	1N NaOH 8°C 3 h	NR	30% v/v H_2O_2 8°C 1 h	105°C 48 h (In solid state)	UV light 72 h (In solid state)	(Hamdache et al., 2021)
%Degradation	10.68% 10.72%	14.57% 16.12%	NR NR	0.31% 0.27%	0.55% 0.67%	0.94% 0.84%	

[1]NR: Not Reported [2]ND: No Degradation

Summary of studies conducted with SOF in combination with VEL

Thirteen studies have designed as stability indicating analysis method for SOF and VEL, simultaneously (Bandla & Ganapaty, 2017; Damle & Kalaskar, 2020; Godela & Sowjanya, 2020; Harshalatha et al., 2018; Hemchand et al., 2018; Lakshmana Rao & Pallavi, 2019; Lakshmi et al., 2018; Namratha & Vijayalakshmi, 2021; Priyanka et al., 2018; Rao et al., 2018; Saroja et al., 2018; Susmita & Rajitha, 2018; Zaman & Hassan, 2021).

In a study by Damle et al., a stability-indicating HPTLC method for SOF and VEL was designed and validated according to the ICH guidelines with a shorter run-time than previously reported methods (Damle & Kalaskar, 2020). Lakshmana Rao et al., developed and validated an accurate, simple stability-indicating RP-HPLC method for estimating SOF and VEL in tablet form with short retention times suitable for quality control testing (Lakshmana Rao & Pallavi, 2019). A stability-indicating RP-HPLC method was developed to estimate SOF and VEL in tablets to separate the compounds and their DP (Saroja et al., 2018). In another study, RP-HPLC, UPLC, and a new stability-indicating RP-UFLC method were designed to determine SOF and VEL in tablets, and stress studies were also conducted (Hemchand et al., 2018). Bandla et al., developed a stability-indicating RP-HPLC method for rapid simultaneous quantification of SOF and VEL in the final product (Bandla & Ganapaty, 2017). A simple and specific stability-indicating RP-HPLC method was designed to simultaneously determine SOF and VEL, which exhibited a much shorter retention time than previously reported methods (Rao et al., 2018). Lakshmi et al., reported the development of a stability-indicating RP-UPLC method for the simultaneous estimation of SOF and VEL, which was accurate, rapid, simple, and economical (Lakshmi et al., 2018). A stability-indicating RP-HPLC designed for the simultaneous determination of SOF and VEL in bulk form, which can also be used for the routine analysis of two drugs in pharmaceutical products, was reported by Priyanka et al. (Priyanka et al., 2018). Zaman et al. developed an accurate and simple stability-indicating HPLC-UV method for analyzing process impurities and DP of SOF and VEL in pharmaceutical formulations and characterized the DP (Zaman & Hassan, 2021). A rapid and simple stability-indicating RP-HPLC method for the simultaneous determination of SOF and VEL in tablet form was developed by Harshalata et al. (Harshalatha et al., 2018) as well as an accurate and simple stability-indicating UPLC method for the simultaneous estimation of SOF and VEL in tablets has been reported by Susmita et al. (Susmita & Rajitha, 2018). Namratha et al. have also designed a stability-indicating UPLC method for the simultaneous estimation of SOF and VEL in tablets (Namratha & Vijayalakshmi, 2021). Godela et al., reported sensitive, simple and specific stability- indicating RP-HPLC method for the simultaneous estimation of SOF and VEL in bulk form, which was useful for monitoring the quality of these two drugs (R Godela & Sowjanya, 2020).

The conditions and results of the stress tests used are listed in (Tables 2 and 3), respectively. In Studies No. 1, 8 and 13, stress tests were performed on the bulk form. The rest of the tests were performed on tablet dosage forms.

Table 2. Summary of stress studies of SOF and VEL simultaneously

Study (Form)	Hydrolysis			Oxidation	Thermal	Photolytic	Reference
	Acidic	Basic	Neutral				
Study 1 (Bulk)	0.1N HCl Immediately	0.1N NaOH 20 min	Water Immediately	30% H_2O_2 1h	80°C 4 h (In solid state)	Fluorescence (1.2 million lux hrs/$m^{2)}$ or UV light (200-watt-hrs/m^2) (In solid state)	(Damle & Kalaskar, 2020)
Study 2 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 1 h	20% H_2O_2 60°C 30 min	105°C 1 h (In solution state)	UV light in UV chamber 1 day or 200-watt hrs/m^2 (In solution state)	(Lakshmana Rao & Pallavi, 2019)
Study 3 (Tablet)	0.1N HCl 25°C 30 min	0.1N NaOH 25°C 30 min	NR	30% H_2O_2 25°C 30 min	105°C 30 min (In solution state)	Sunlight 1 day (In solution state)	(Saroja et al., 2018)
Study 4 (Tablet)	0.1N HCl 60°C 30 min	0.1N NaOH 60°C 2 min	NR	30% H_2O_2 40°C 1 h	60°C 1 h (In solution state)	UV light in a photostability chamber 48 h (In solid state)	(Hemchand et al., 2018)
Study 5 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 6 h	H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light 7 days or 200-watt hrs/m^2 (In solution state)	(Bandla & Ganapaty, 2017)
Study 6 (Tablet)	2N HCl 60°C 30 min In dark	2N NaOH 60°C 30 min In dark	Water 60°C 30 min In dark	20% H_2O_2 25°C 1 day In dark	105°C 1 h (In solid state)	UV light in UV chamber 1 day or 200-watt hrs/m^2 (In solution state)	(Rao et al., 2018)
Study 7 (Tablet)	0.1N HCl 60°C 24 h	0.1N NaOH 60°C 24 h	NR	12.5% H_2O_2 25°C 15 min	110°C 3 h (In solid state)	UV light in UV chamber 24 h (In solution state)	(Lakshmi et al., 2018)
Study 8 (Bulk)	NR	NR	NR	NR	NR	NR	(Priyanka et al., 2018)
Study 9 (Tablet)	0.1N HCl 25°C 8 h	0.1N NaOH 25°C 8 h	NR	3% H_2O_2 25°C 7 days	105°C 8 h (In solid state)	UV light 25°C 7 days or Fluorescent light 1.2 million lux hrs/m^2 7 days (In solid state)	(Zaman & Hassan, 2021)
Study 10 (Tablet)	0.1N HCl 85°C 5 h	0.1N NaOH 85°C 6 h	NR	3% H_2O_2 55°C 8 h	85°C 30 h (In solid state)	UV degradation in 256 nm 30 h (In solution state)	(Harshalatha et al., 2018)
Study 11 (Tablet)	NR	NR	NR	NR	NR	NR	(Susmita & Rajitha, 2018)
Study 12 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 6 h	20% H_2O_2 60°C 30 min	105°C 6 h (In solution state)	NR	(Namratha & Vijayalakshmi, 2021)
Study 13 (Bulk)	0.1N HCl 70°C 24 h	0.1N NaOH 70°C 24 h	NR	3% H_2O_2 70°C 24 h	80°C 24 h (In solution state)	UV light 24 h (In solution state)	(Godela & Sowjanya, 2020)

Table 3. Summary % degradation results for SOF and VEL tested simultaneously

Study		Hydrolysis			Oxidation	Thermolysis	Photolysis	Reference
		Acidic	Basic	Neutral				
Study 1	SOF	16.3	16.46	17.34	21.97	18.67	UV:25.98 Fluorescence:12.99	(Damle & Kalaskar, 2020)
	VEL	23.96	7.84	7.78	14.05	11.87	UV:20.75 Fluorescence:6.76	
Study 2	SOF	4.66	4.04	2.83	3.77	2.86	2.83	(Lakshmana Rao & Pallavi, 2019)
	VEL	4.92	4.24	0.94	3.18	2.97	2.68	
Study 3	SOF	7.45	7.16	NR	7.39	6.45	6.73	(Saroja et al., 2018)
	VEL	6.80	6.19	NR	5.88	7.21	6.27	
Study4	SOF	3.87	71.06	NR	5.07	5.02	4.04	(Hemchand et al., 2018)
	VEL	0.16	0.09	NR	2.99	0.61	0.22	
Study5	SOF	4.79	2.79	0.81	1.96	0.84	0.59	(Bandla & Ganapaty, 2017)
	VEL	4.97	2.66	0.96	1.67	0.51	0.76	
Study6	SOF	3.49	3.49	0.84	3.61	4.57	1.79	(Rao et al., 2018)
	VEL	3.64	3.64	0.90	2.90	4.11	2.44	
Study7	SOF	5.60	5.20	NR	5.30	3.00	5.5	(Lakshmi et al., 2018)
	VEL	6.50	5.20	NR	5.80	2.00	7.00	
Study8	SOF	3.78	3.77	NR	5.69	4.37	4.38	(Priyanka et al., 2018)
	VEL	5.17	5.00	NR	5.80	2.51	3.19	
Study9	SOF	5.88	85.64	NR	3.48	1.82	UV:0.33 Fluorescence:0.14	(Zaman & Hassan, 2021)
	VEL	1.84	1.03	NR	18.40	2.09	UV:1.25 Fluorescence:0.90	
Study10	SOF	12.76	10.82	NR	11.26	7.30	8.74	(Harshalatha et al., 2018)
	VEL	12.41	10.74	NR	6.24	8.21	8.30	
Study11	SOF	3.56	2.10	NR	0.95	0.05	-0.19	(Susmita & Rajitha, 2018)
	VEL	5.42	3.23	NR	2.34	1.14	1.29	
Study12	SOF	5.09	4.33	0.23	2.59	2.21	NR	(Namratha & Vijayalakshmi, 2021)
	VEL	7.18	5.60	0.67	2.79	1.93	NR	
Study 13	SOF	22.00	18.40	NR	19.50	0.85	0.45	(Godela & Sowjanya, 2020)
	VEL	13.60	12.70	NR	12.70	4.20	0.21	

Summary of studies conducted on SOF and DAC

Two studies of SOF and DAC were identified (Bandla & Ganapaty, 2018; Ramreddy Godela & Sowjanya, 2021) and the results are summarized in (Tables 4. and 5.).

In a study conducted by Bandla et al., a stability-indicating RP-HPLC method was developed for the simultaneous estimation of SOF and DAC in the bulk form in which stress studies were also conducted has been reported (Bandla & Ganapaty, 2018). A stability-indicating RP-HPLC for the simultaneous determination of SOF and DAC in the bulk form in which the well-resolved separation of SOF and DAC from their DP has been reported and can be used to analyze these two drugs by the pharmaceutical industry (Ramreddy, Godela & Sowjanya, 2021).

Table 4. Summary of stress studies conducted with SOF and DAC simultaneously.

Study (Form)	Hydrolysis			Oxidation	Thermal	Photolytic	Reference
	Acidic	Basic	Neutral				
Study 1 (Bulk)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 6 h	20% H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light in UV chamber 7 days Or 200-watt hours/m^2 (In solution state)	(Bandla & Ganapaty, 2018)
Study 2 (Bulk)	0.1N HCl 70°C 2 h	0.1N NaOH 70°C 2 h	NR	3% H_2O_2 70°C 2 h	In a hot air oven at 80°C/75% RH 24 h[1] (In solution state)	NR	(Ramreddy Godela & Sowjanya, 2021)

[1] The problem in this part of the study is that there is no need to control the humidity in the solution state.

The summary of stress studies results of SOF and DAC is given in (Table 5.).

Table 5. Summary of % degradation results of SOF and DAC stress testing

Study		Hydrolysis			Oxidation	Thermolysis	Photolysis	Reference
		Acidic	Basic	Neutral				
Study 1	SOF	4.42	5.25	0.77	7.92	2.23	1.71	(Bandla & Ganapaty, 2018)
	DAC	4.26	4.41	0.15	5.09	2.02	1.07	
Study 2	SOF	15.6	51.20	NR	2.00	1.20	NR	(Ramreddy Godela & Sowjanya, 2021)
	DAC	9.80	16.40	NR	7.00	0.60	NR	

Summary of studies conducted on SOF, VEL and VOX

Five combination studies, which included SOF, VEL and VOX, were identified (Balaswami et al., 2018; Deepthi & Sankar, 2020; Kokkirala & Suryakala, 2020; Lakshmi Maneka S et al., 2020; Padmini M et al., 2019) and the conditions and results of stress testing are summarized in (Tables 6. and 7.).

A stability-indicating RP-HPLC developed for the determination of SOF, VEL, and VOX in the tablets was reported to be rapid and suitable for the routine analysis and quality control of these drugs (Deepthi & Sankar, 2020). A simple, rapid and linear stability indicating RP-HPLC method for estimating SOF, VEL, VOX and DP simultaneously in bulk has also been reported (Kokkirala & Suryakala, 2020). Balaswami et al., reported a simple and economic stability-indicating RP-HPLC method for the simultaneous estimation of SOF, VEL and VOX which was applied to routine analysis of these three drugs in the bulk form (Balaswami et al., 2018). Padmini et al., reported stability- indicating RP-HPLC method for the simultaneous estimation of SOF, VEL, and VOX in the bulk form (Padmini M et al., 2019). A stability-indicating RP-UPLC for the simultaneous estimation of SOF, VEL, and VOX in the bulk form with much shorter retention and run times when compared to conventional HPLC methods was reported (Lakshmi Maneka S et al., 2020).

It is worth noting that Studies No. 2 -5 were undertaken using bulk API, whereas Study No. 1 used the tablet form.

Table 6. Summary of stress study conditions for SOF, VEL and VOX

Study (Form)	Hydrolysis			Oxidation	Thermolysis	Photolysis	Reference
	Acidic	Basic	Neutral				
Study 1 (Tablet)	2 N HCl 60°C 30 min	2 N NaOH 60°C 30 min	Water 60°C 6 h	20% H_2O_2	105°C 6 h (In solution state)	UV light 7 days Or 200-watt hours/m^2 (In solution state)	(Deepthi & Sankar, 2020)
Study 2 (Bulk)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 6 h	20% H_2O_2 60°C 30 min	105°C 1 h (In solution state)	UV light 1 day Or 200-watt hours/m^2 (In solution state)	(Kokkirala & Suryakala, 2020)
Study 3 (Bulk)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 6 h	20% H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light 7 days Or 200-watt hours/m^2 3 days (In solution state)	(Balaswami et al., 2018)
Study 4 (Bulk)	1N HCl 60°C 30 min	1N NaOH 60°C 30 min	NR	20% H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light 3 days Or 200-watt hours/m^2 (In solution state)	(Padmini M et al., 2019)
Study 5 (Bulk)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 30 min	20% H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light 3 days Or 200-watt hours/m^2 (In solution state)	(Lakshmi Meneka S et al., 2020)

Table 7. % Degradation data following stress testing of SOF, DAC and VOX in combination

Study		Hydrolysis			Oxidation	Thermolysis	Photolysis	Reference
		Acidic	Basic	Neutral				
Study 1	SOF	5.77	4.65	0.63	4.05	1.75	2.27	(Deepthi & Sankar, 2020)
	VEL	5.96	4.90	0.49	4.42	3.57	2.42	
	VOX	5.86	5.51	0.33	3.39	1.29	0.92	
Study 2	SOF	Two DPs	One DP	ND	Two DPs	ND	ND	(Kokkirala & Suryakala, 2020)
	VEL	Two DPs	One DP	ND	Two DPs	ND	ND	
	VOX	Two DPs	One DP	ND	Two DPs	ND	ND	
Study 3	SOF	95.50	94.60	0.90	3.84	4.40	3.00	(Balaswami et al., 2018)
	VEL	96.23	95.92	0.48	2.84	1.88	1.24	
	VOX	95.50	94.60	0.90	3.43	4.40	3.00	
Study 4	SOF	8.90	7.97	0.67	6.11	3.84	1.56	(Padmini M et al., 2019)
	VEL	4.39	3.55	0.55	3.06	2.37	1.51	
	VOX	7.72	6.39	0.75	4.54	2.31	1.28	
Study 5	SOF	5.90	4.44	0.58	3.19	2.88	1.18	(Lakshmi Meneka S et al., 2020)
	VEL	5.83	4.31	0.90	3.10	2.26	1.58	
	VOX	4.72	3.64	0.32	3.58	2.96	1.51	

Summary of studies conducted on SOF and LED

Fifteen studies in which a combination of SOF and LED were analyzed were identified and included (Bhavani & Maduri, 2020; El-Waey et al., 2023; El-Yazbi et al., 2020; Hassouna et al., 2017; Jahnavi & Ganapaty, 2018; Kumar & Rao, 2018; Kumari & Sankar, 2019; Mankar et al., 2019; Mastanamma et al., 2018; Narla & Pappula, 2020; Rao et al., 2017; Reddy et al., 2018; Rote et al., 2017; Suganthi et al., 2019;

Veereswara Rao et al., 2018; Yeram et al., 2019).

In a study conducted by Hassouna et al. assay and dissolution methods were developed to determine LED and SOF in bulk. Furthermore, stress studies were undertaken to develop and validate a RP-HPLC method to determine LED selectively and SOF with precision (Hassouna et al., 2017). Rao et al. reported a precise and accurate RP-HPLC method that had been developed for the determination of LED in tablets and forced degradation studies were performed using the conditions recommended in the ICH guideline and photodiode array detection (PDA) used to monitor the API and impurities (Rao et al., 2017). A RP-HPLC stability-indicating method to determine LED and SOF in the bulk form with forced degradation studies was performed according to ICH guidelines (Rote et al., 2017). The study conducted by Mastanamma et al. aimed to develop an analytical method for the simultaneous estimation of LED and SOF in the presence of DP produced under stress conditions using RP-HPLC with UV detection (Mastanamma et al., 2018). Kumar et al. aimed to develop a stability-indicating RP-HPLC method to determine LED and SOF in tablets using ICH guidelines (Kumar & Rao, 2018), whereas Veereswara reported a new stability-indicating RP-HPLC method for the determination of LED and SOF in tablets (Veereswara Rao et al., 2018). Bandla et al. reported a RP-HPLC stability-indicating method for determining LED and SOF in tablets in the presence of DP, produced under stress conditions (Jahnavi & Ganapaty, 2018). A precise, simple, and stability-indicating RP-HPLC method was developed to estimate LED and SOF in tablets, and the method was validated as recommended in the ICH guidelines (Reddy et al., 2018).

By 2019 a simple, robust and selective RP-HPLC method for the estimation of LED and SOF in bulk had been developed using a QbD approach and forced degradation studies were applied for the purposes of method optimization (Yeram et al., 2019). An accurate, precise, simple RP-HPLC method was developed to estimate LED and SOF in tablets and forced degradation studies, applied according to ICH guidelines, and revealed the final method was stability-indicating (Mankar et al., 2019). In a study conducted by Kumari et al., a stability-indicating UPLC method was developed and validated using the ICH guidelines, and the method was faster, more accurate, and more precise when compared to other methods. All samples subjected to stress conditions were analyzed using the optimized method, and based on the ICH guidelines, the method was found to be stability-indicating (Kumari & Sankar, 2019). Bhavani et al. reported that no stability-indicating method existed for the analysis of LED and SOF in multi-component tablets, and the primary purpose of their study was to develop a stability-indicating method for the analysis of LED and SOF in bulk. The RP-HPLC method using photodiode array detection was developed and optimized with the aid of forced degradation studies, which resulted in a highly specific approach to analysis (Bhavani & Maduri, 2020). In a study conducted by Suganthi et al., it was reported that no stability-indicating method had been published to determine LED and SOF in tablets. Therefore, their study aimed to develop a rapid, simple, and robust HPLTC method for the assay of LED and SOF in tablets, and forced degradation studies were performed according to ICH guidelines (Suganthi et al., 2019). El Yazbi et al. reported a precise, rapid, simple, and eco-friendly HPTLC method for the simultaneous analysis of LED and SOF in tablets. Forced degradation studies performed according to ICH guidelines under acidic and alkaline hydrolysis, oxidative, and photolytic conditions revealed the method was stability-indicating (El-Yazbi et al., 2020). Narla et al. developed a stability-indicating UHPLC method for the simultaneous estimation of SOF and LED in tablets. The proposed method is simple and accurate and was used to analyze tablets in the presence of DP (Narla & Pappula, 2020).

A summary of the stress test conditions and % degradation is reported in (Tables 8. and 9.), respectively. In studies No. 1, 3, 4, 9, and 12, forced degradation studies were performed on bulk API, whereas in all other studies, forced degradation studies were undertaken using tablets.

Table 8. Summary of stress study conditions for SOF and LED

Study (Form)	Hydrolysis			Oxidation	Thermolysis	Photolysis	Reference
	Acidic	Basic	Neutral				
Study 1 (Bulk)	1N HCl 25°C 48 h	1N NaOH 25°C 48 h	NR	3%H_2O_2 25°C 48 h	80°C 8 h in dry heat or Stability chamber 40°C±2,75%±5%RH 7 days in wet heat (In solid state)	Sunlight 48 h (In solid state)	(Hassouna et al., 2017)
Study 2 (Tablet)	1.1N HCl 25°C 30 min	1.1N NaOH 25°C 30 min	NR	3%H_2O_2 25°C 30 min	105°C 30 min (In solid state)	Sunlight 24 h (In solid state)	(Rao et al., 2017)
Study 3 (Bulk)	1.1N HCl 25°C 24 h	1.1N NaOH 25°C 24 h	Water 25°C 24 h	3%H_2O_2 25°C 24 h	NR	NR	(Rote et al., 2017)
Study 4 (Bulk)	5N HCl 60°C 30 min	5N NaOH 70°C 60 min	Water 70°C 3 h	30%H_2O_2 70°C 1 h	105°C 72 h (In solid state)	UV light 1.2 million lux hours 24 h (In solid state)	(Mastanamma et al., 2018)
Study 5 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 6 h	20%H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV chamber 7 days or 200-watt hours/ m^2 (In solution state)	(Kumar & Rao, 2018)
Study 6 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 1 h	20% H_2O_2 60°C 30 min	105°C 6 h in dry heat Stability chamber 40±2°C,75%±5%RH 1 day in wet heat. (In solution state)	UV light (In solution state)	(Veereswara Rao et al., 2018)
Study 7 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	NR	20% H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light 7 days or 200-watt hours/ m^2 (In solution state)	(Jahnavi & Ganapaty 2018)
Study 8 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	NR	20%H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light 254nm 7 days or 200-watt hours/m^2 (In solution state)	(Reddy et al., 2018)
Study 9 (Bulk)	1N HCl 80°C 1 h	1N NaOH 80°C 1h	NR	3%H_2O_2 25°C 25 min	80°C 2 h (In solution state)	UV light 290nm 7 days (In solid state)	(Yeram et al., 2019)
Study 10 (Tablet)	5N HCl 60°C 30 min	5N NaOH 70°C 60 min	NR	30%H_2O_2 70°C 48 h	105°C 48 h (In solution state)	UV light 1.2 million lux hours 48 h (In solution state)	(Mankar et al., 2019)
Study 11 (Tablet)	2N HCl 60°C 30 min	2N NaOH 60°C 30 min	Water 60°C 6 h	20%H_2O_2 60°C 30 min	105°C 6 h (In solution state)	UV light 7 days (In solution state)	(Kumari & Sankar, 2019)
Study 12 (Bulk)	1N HCl 70°C 48 h	1N NaOH 70°C 48 h	NR	3%H_2O_2 70°C 48 h	70°C 14 days (In solid state)	UV light 14 days (In solid state)	(Bhavani & Maduri, 2020)
Study 13 (Tablet)	0.1 N HCl 80°C 5 h	0.1 N NaOH 80°C 5 h	Water 80°C 5 h	6% H_2O_2 25°C 5 h	80°C 5 h (In solid state)	UV light 5 h (In solid state)	(Suganthi et al., 2019)
Study 14 (Tablet)	1N HCl 90°C 1 h	1N NaOH 90°C 1 h	NR	30% H_2O_2 80°C 1h	NR	UV light 254 nm for 12 h UV light 365 nm for 12 h (In solution state)	(El-Yazbi et al., 2020)
Study 15 (Tablet)	1N HCl 25°C 2 h	1N NaOH 25°C 2 h	NR	30% H_2O_2 25°C 2 h	110°C 2 h (In solution state)	Sunlight 8 h (In solution state)	(Narla & Pappula, 2020)

Table 9. % Degradation following analysis simultaneous analysis of SOF and LED

Study		Hydrolysis			Oxidation	Thermolysis	Photolysis	Reference
		Acidic	Basic	Neutral				
Study 1	SOF	12.44	12.60	NR	5.40	Dry: 7.89 Wet: 12.54	6.36	(Hassouna et al., 2017)
	LED	10.32	10.05	NR	1.60	Dry: 6.12 Wet: 10.6	4.63	
Study 2	SOF	2.27	2.14	NR	1.67	1.74	1.39	(Rao et al., 2017)
	LED	1.97	1.71	NR	1.92	1.06	1.32	
Study 3	SOF	4.03	1.35	2.15	0.35	NR	NR	(Rote et al., 2017)
	LED	0.08	0.99	0.22	0.22	NR	NR	
Study 4	SOF	29.7	28.1	24.00	26.4	20.00	24.10	(Mastanamma et al., 2018)
	LED	26.40	29.50	28.30	26.00	24.80	25.10	
Study 5	SOF	2.97	2.17	0.16	1.65	0.85	0.38	(Kumar & Rao, 2018)
	LED	4.72	2.29	0.19	1.24	0.85	0.61	
Study 6	SOF	5.41	3.67	2.69	2.88	Dry: 1.15 Wet: 3.75	0.87	(Veereswara 2018)
	LED	4.75	4.20	2.57	5.95	Dry: 2.00 Wet: 3.5	1.00	
Study 7	SOF	4.90	3.03	NR	1.30	0.70	0.48	(Jahnavi & Ganapaty, 2018)
	LED	3.65	3.26	NR	2.25	1.09	0.94	
Study 8	SOF	5.67	3.93	NR	3.35	2.67	1.70	(Reddy et al., 2018)
	LED	5.05	4.43	NR	3.66	2.99	1.83	
Study 9	SOF	3.10	7.80	NR	1.40	3.20	0	(Yeram et al., 2019)
	LED	17.13	12.45	NR	11.91	9.20	18.72	
Study 10	SOF	0.44	0.02	NR	0.41	0.04	0.01	(Mankar et al., 2019)
	LED	1.18	0.05	NR	1.73	0.05	0.07	
Study 11	SOF	6.09	5.49	0.72	3.83	2.69	1.99	(Kumari & Sankar, 2019)
	LED	6.21	4.84	0.83	3.14	2.35	1.52	
Study 12	SOF	NR	NR	NR	NR	NR	NR	(Bhavani & Maduri, 2020)
	LED	NR	NR	NR	NR	NR	NR	
Study 13	SOF	NR	NR	NR	NR	NR	NR	(Suganthi et al., 2019)
	LED	NR	NR	NR	NR	NR	NR	
Study 14	SOF	52.29	58.96	NR	16.90	NR	0	(El-Yazbi et al., 2020)
	LED	10.96	8.57	NR	37.99	NR	0	
Study 15	SOF	7.72	9.55	NR	8.72	8.03	8.03	(Narla & Pappula, 2020)
	LED	5.41	5.35	NR	5.64	4.45	4.70	

The summary of chromatographic conditions used in all studies is listed in (Table 10.).

Table 10. Summary of chromatographic conditions used in all studies

Study		Method	Mobile Phase and Elution Mode	Stationary Phase	Detector Wavelength (nm)	LOQ (µg/ml)	Reference
SOF ONLY	1	RP-UFLC	0.1% FA[1]: ACN[2] (40: 60% v/v)	C8	PDA[3], 259	0.76	(Annapurna et al., 2018)
	2	RP-HPLC	Methanol: Water (70: 30% v/v)	C18	UV[4], 254	1.00	(Nebsen & Elzanfaly, 2016)
	3	UPLC	ACN: 0.1% FA	C18	PDA, 260	0.83	(Pottabathini et al., 2016)
	4	HPLC	AA[5]:ACN	C18	PDA, 260	NR	(Swain et al., 2016)
	5	RP-HPLC	Methanol: Water (65: 35% v/v)	C18	PDA, 261	NR	(Vanitha et al., 2018)
	6	RP-HPLC	Methanol: Water with 0.1% FA (50:50 %v/v)	C18	PDA, 261	NR	(Agarwal et al., 2022)
	7	RP-HPLC	PDP[6]: ACN (60:40 %v/v)	C18	PDA, 260	30.62	(Hassouna &Mohamed., 2018)
	8	UPLC	0.1% OPA[7]: Methanol (40:60% v/v)	C18	UV, 260	0.55	(Abdel-Razeg et al., 2019)
	9	RP-HPLC	0.1% OPA: ACN (30:70% v/v)	C18	UV, 260	1.07	(Lalitha et al., 2018)
	10	RP-HPLC	0.05% PA[8]: ACN	C18	PDA, 260	1.45	(Hamdache et al., 2021)
SOF&VEL	1	HPTLC	EA[9]: IPA[10] (9:1 %v/v)	Silica gel 60 F 254	UV, SOF: 260 VEL:302	SOF: 76.25 ng/band VEL: 30.19 ng/band	(Damle & Kalaskar, 2020)
	2	RP-HPLC	ODP[11] (0.01%): ACN (50:50% v/v)	C18	UV, 240	SOF: 1.32 VEL: 1.01	(Lakshmana Rao & Pallavi, 2019)
	3	RP-HPLC	PDP: Methanol (60:40% v/v)	C18	PDA, 240	SOF: 1.60 VEL: 0.62	(Saroja et al., 2018)
	4	RP-HPLC	0.1% FA:ACN	C8	PDA, 259	SOF: 3.83 VEL: 0.91	(Hemchand et al., 2018)
	5	RP-HPLC	PDP: ACN (50:50% v/v)	C18	PDA, 240	SOF: 0.78 VEL: 0.50	(Bandla & Ganapaty, 2017)
	6	RP-HPLC	PDP: ACN (50:50% v/v)	C8	PDA, 240	SOF: 0.61 VEL: 0.65	(Rao et al., 2018)
	7	RP-UPLC	Phosphate buffer: ACN (50:50% v/v)	C18	PDA, 240	SOF: 4.49 VEL: 5.13	(Lakshmi et al., 2018)
	8	RP-HPLC	PDP: ACN (50:50% v/v)	YMC Column	UV, 255	SOF: 1.20 VEL: 0.30	(Priyanka et al., 2018)
	9	HPLC	AA[12]: ACN (45:55% v/v)	C18	UV, 268	SOF: 0.38 VEL: 0.24	(Zaman & Hassan, 2021)
	10	RP-HPLC	SDOP[13]: ACN (85:15% v/v)	C18	PDA, 292	SOF: 0.04 VEL: 0.06	(Harshalatha et al., 2018)
	11	UPLC	PDP: ACN (45:55% v/v)	C18	UV, 250	SOF: 0.35 VEL: 0.03	(Susmita & Rajitha, 2018)
	12	UPLC	PPM[14]: ACN (50:50% v/v)	C8	PDA, 260	SOF: 0.29 VEL: 1.25	(Namratha & Vijayalakshmi, 2021)
	13	HPLC	FA in Water: ACN: Methanol (30:30:40% v/v)	Phenyl XDB	PDA, 273	SOF: 1.20 VEL: 0.30	(R Godela & Sowjanya, 2020)
SOF & DAC	1	RP-HPLC	PDP: ACN (50:50% v/v)	C18	PDA, 254	SOF: 0.07 DAC: 0.03	(Bandla & Ganapaty, 2018)
	2	RP-HPLC	ACN: TFA[15] in water (50:50% v/v)	XDB Phenyl	PDA, 275	SOF: 15.80 DAC: 7.80	(Ramreddy Godela & Sowjanya, 2021)

SOF&VEL&VOX	1	RP-HPLC	OPA: ACN (55:45% v/v)	C18	PDA, 220	SOF: 0.34 VEL: 0.29 VOX: 0.17	(Deepthi & Sankar, 2020)
	2	RP-HPLC	SDOP: ACN (60:40% v/v)	C18	PDA, 220	SOF: 1.14 VEL: 0.99 VOX: 0.24	(Kokkirala & Suryakala, 2020)
	3	RP-HPLC	OPA: ACN (50:50% v/v)	C18	PDA, 220	SOF: 0.25 VEL: 0.87 VOX: 0.31	(Balaswami et al., 2018)
	4	RP-HPLC	ACN: Water (65:35% v/v)	C18	PDA, 220	SOF: 2.32 VEL: 0.53 VOX: 0.70	(Padmini M et al., 2019)
	5	RP-UPLC	PDP: Methanol (50:50% v/v)	C18	PDA, 260	SOF: 0.02 VEL: 0.40 VOX: 0.02	(Lakshmi Meneka S et al., 2020)
SOF&LED	1	RP-HPLC	Phosphate buffer: ACN (50:50% v/v)	C18	UV, 254	SOF: 12.54 LED: 11.03	(Hassouna et al., 2017)
	2	RP-HPLC	DHP[16]: ACN (60:40% v/v)	C18	PDA, 282	SOF: 0.75 LED: 0.25	(Rao et al., 2017)
	3	RP-HPLC	Methanol: Water with 0.05% acetic acid (83:17% v/v)	Purospher RP-18	UV, 245	SOF: 10.19 LED: 3.30	(Rote et al., 2017)
	4	RP-HPLC	ACN: TEA[17] (50:50% v/v)	C18	UV, 227	SOF: 0.50 LED: 0.51	(Mastanamma et al., 2018)
	5	RP-HPLC	ACN: 0.1% OPA buffer (35:65% v/v)	C18	UV, 272	SOF: 3.70 LED: 0.56	(Kumar & Rao, 2018)
	6	RP-HPLC	ACN: 0.1% OPA (50:50% v/v)	C8	UV, 230	SOF: NR LED: NR	(Veereswara Rao et al., 2018)
	7	RP-HPLC	ACN: 0.1% OPA (55:45% v/v)	C18	PDA, 270	SOF: 0.65 LED: 0.19	(Jahnavi & Ganapaty, 2018)
	8	RP-HPLC	ACN: OPA (55:45% v/v)	C8	PDA, 260	SOF: 0.76 LED: 1.13	(Reddy et al., 2018)
	9	RP-HPLC	Methanol: AA with GAA[18] (70:30% v/v)	C18	PDA, 254	SOF: 1.50 LED: 11	(Yeram et al., 2019)
	10	RP-HPLC	TFA: ACN (70:30% v/v)	C18	UV, 245	SOF: 1.20 LED: 0.40	(Mankar et al., 2019)
	11	UPLC	PDP: ACN (50:50% v/v)	C18	UV, 220	SOF: 3.91 LED: 1.29	(Kumari & Sankar, 2019)
	12	RP-HPLC	OPA: Methanol (45:55% v/v)	C8	PDA, 238	SOF: 2.21 LED:0.70	(Bhavani & Maduri, 2020)
	13	HPTLC	Hexane: EA: Methanol (5:3:2 %v/v)	Silica gel 60 F_{254}	UV, 288	SOF: 1.32 ng/spot LED: 0.40 ng/spot	(Suganthi et al., 2019)
	14	HPTLC	EA: Methanol: Water: GAA (30:1.5:1:0.2 %v/v)	Silica gel F_{254}	PDA, SOF 260 LED 320	SOF: 1.90 µg/band LED: 0.33 µg/band	(El-Yazbi et al., 2020)
	15	RP-HPLC	ACN: Phosphate (55:45% v/v)	C18	PDA, 247	SOF: 0.28 LED: 0.32	(Narla & Pappula, 2020)

[1]FA: Formic Acid
[2]ACN: Acetonitrile
[3]PDA: Photo Diode Array
[4]UV: Ultra Violet
[5]AA: Acetic Acid
[6]PDP: Potassium Dihydrogen Phosphate
[7]OPA: Ortho Phosphoric Acid
[8]PA: Phosphoric Acid
[9]EA: Ethyl Acetate
[10]IPA: Iso Propyl Alcohol
[11]ODP: Ortho Dihydrogen Phosphate
[12]AA: Ammonium Acetate
[13]SDOP: Sodium Dihydrogen Orthophosphate
[14]PPM: Potassium Phosphate Monobasic
[15]TFA: Trifluoro Acetic Acid
[16]DHP: Disodium Hydrogen Phosphate
[17]TEA: Triethylamine
[18]GAA: Glacial Acetic Acid

Analysis of SOF alone

Ten studies in which the analysis of SOF alone with stress testing and designated stability-indicating method for the drug was identified and included (Abdel-Razeq et al., 2019; Agarwal et al., 2022; Annapurna et al., 2018; Hamdache et al., 2021; Hassouna & Mohamed, 2018; Lalitha et al., 2018; Nebsen & Elzanfaly, 2016; Pottabathini et al., 2016; Swain et al., 2016; Vanitha et al., 2018).

In Study No. 1 (Annapurna et al., 2018), the conditions used for stress testing were not based on those recommended in the ICH guidelines (Guideline, 2003). The API was tested under extremely severe stress conditions for a short period. By way of example, the concentrations of H_2O_2 were 30% v/v, and the temperature was 60°C for the oxidative stress test. However, oxidative stress testing with H_2O_2 should be performed at or lower than room temperature as at high temperatures, H_2O_2 decomposes to form hydroxyl radicals, which are highly reactive in their own right and may result in the formation of DPs that would never form under normal storage conditions in bulk and pharmaceutical dosage forms (Yaripour, Rashid, Alibakhshi, & Mohammadi, 2015). Therefore, when severe stress conditions are applied over a period of time, the API may be destroyed instead of undergoing degradation. This study stated that the API was sensitive to alkaline hydrolytic and oxidative stress conditions but did not degrade under thermal and photolytic conditions. Different results may have been observed if the conditions and duration of stress were selected based on the relevant guidelines. In Study No. 2 (Nebsen & Elzanfaly, 2016), the duration of alkaline hydrolysis was not selected according to the published guidelines and 90% and 100% of API degraded in 6 and 24 hours, respectively. The ICH guideline states that the duration of stress testing should be selected such that a maximum of 5-20% of API degrades during the test period (Baertschi, Alsante, & Reed, 2016). In addition, for the thermal studies, the API was tested at 80°C while the recommendation is that the temperature range used fall between 50 and 70°C (Baertschi et al., 2016). It was reported that the API did not degrade under this condition. However, the thermal study test period should be extended to ensure the API is or is not sensitive to the test conditions. According to the ICH guideline, the recommended concentration of H_2O_2 should be between 0.3-3% v/v (Baertschi et al., 2016), whereas in this study, 3% and 6% v/v concentrations were used. Consequently, the DP produced at the 6% v/v concentration may not be reliable. Furthermore, the ICH guideline states that photolytic studies should be performed in the presence of UV and visible light (Baertschi et al., 2016), but in this study, the API was only exposed to UV light for 6 hours in an aqueous solution of the API which turned yellow in these conditions with an apparent 70% degradation, suggesting the study is incomplete and possibly unreliable. In Study No. 3 (Pottabathini et al., 2016), a temperature of 80°C was used to perform acid hydrolysis testing of SOF, which is higher than that recommended in guidelines which require testing at room temperature or temperatures up to a maximum of 70°C (Baertschi et al., 2016). In addition, 30% v/v H_2O_2 at 80°C was used for the oxidative stress study, which, as previously mentioned, is outside the recommended concentration of H_2O_2 of 0.3-3% v/v at ambient temperature. In Study No. 4 (Swain et al., 2016), the decomposition behavior of API under different stress conditions was monitored using a 30% v/v concentration of H_2O_2 for the oxidative stress study, and a temperature of 80°C was used for the thermal stress test. In Study No. 5 (Vanitha et al., 2018), no decomposition was reported to have occurred when oxidative and thermal stress conditions were used however, the API was found to be sensitive to acidic and alkaline hydrolytic stress conditions. The drawback of these studies is that their duration was too short, and it is unclear whether more decomposition products would have been produced if a longer exposure time had been used. In Study No. 6 (Agarwal et al., 2022), what can be considered acceptable stress conditions were used. For acidic and alkaline hydrolysis, 0.1N HCl

and 0.1N NaOH were used, respectively. The rate of degradation in acidic hydrolysis was remarkable, and a major DP was produced, whereas, during alkaline hydrolysis, 50% of the drug degraded, and another major DP was produced. The conditions used for oxidative stress testing were also acceptable according to guidelines and > 10% API degraded with a major DP produced. The API was stable under thermal and photolytic stress test conditions. It is considered better to select conditions according to approved guidelines to evaluate the effects of light on an API while monitoring the effects of UV light and fluorescence on API degradation. In Study No. 7 (Hassouna & Mohamed., 2018), high concentrations of acid and alkali were used for acid and alkaline hydrolysis experiments. During the oxidation study, a 3% v/v concentration of H_2O_2 was used at room temperature for 48 hours, which resulted in 4.56% degradation. If the duration of this study had been extended, the extent of degradation would have been greater, and the study would have been more accurate. The photolysis study was also conducted for 48 hours, after which 7% degradation had occurred. It would have been better if this study had been continued for a longer time so that the percent degradation would be greater. For thermal studies, the API was exposed to 40°C and 75% humidity in a climatic chamber for one month, which resulted in 4% degradation that suggests the conditions and duration of the study were acceptable. In Study No. 8 (Abdel-Razeg et al., 2019), the API was subjected to acidic, alkaline and oxidative stress conditions. The 5 N concentration of acid and base used was very high. In addition, a very high temperature of 100°C was used for acid and alkaline hydrolysis studies. These conditions are severe and are well beyond those recommended in the guidelines.Furthermore at temperature>70°C, the decomposition kinetics of the API do not follow the Arrhenius model because, at temperatures<70°C, the mechanism of drug degradation may change, and products may be formed that are never formed under normal conditions of drug storage (Yaripour et al., 2015). In Study No. 9 (Lalitha et al., 2018) listed in (Table 1.) the use of mild stress conditions was reported. However, the results suggest the conditions for degradation used were appropriate and degradation between 5-10 % was observed (Baertschi et al., 2016). In Study No. 10 (Hamdache et al., 2021), stress tests were performed on API and tablets. The 1N concentration of acid and alkali used was high. However, the temperature used was 8°C, which is different from the normal storage conditions for the drug. The 30% v/v H_2O_2 used is also high, but the stress was applied at 8°C. The duration of all tests was one hour, which is also different from the conditions for drug storage and is not aligned with the guidelines (Baertschi et al., 2016). The low percent degradation of < 1% under oxidative and photolytic conditions is more than likely due to the short duration of exposure during stress testing.

Analysis of SOF and VEL

The simultaneous stability indicating analysis of SOF and VEL, which included stress testing, was reported in 13 studies (Bandla & Ganapaty, 2017; Damle & Kalaskar, 2020; R Godela & Sowjanya, 2020; Harshalatha et al., 2018; Hemchand et al., 2018; Lakshmana Rao & Pallavi, 2019; Lakshmi et al., 2018; Namratha & Vijayalakshmi, 2021; Priyanka et al., 2018; Rao et al., 2018; Saroja et al., 2018; Susmita & Rajitha, 2018; Zaman & Hassan, 2021).

Different stress test conditions applied to the two compounds resulted in different outcomes (Bandla & Ganapaty, 2017). In addition, the concentration of stress agent used was as recommended in guidelines, and the time of exposure to the stress conditions was more reasonable. However, these studies are not comparable. By way of example, in Study No. 4 (Hemchand et al., 2018), 0.1 N acid and alkali were used however, in Study No. 5 (Bandla & Ganapaty, 2017), a 2 N acid and alkali concentration was used. In both cases, the duration of exposure was 30 min at a temperature of 60°C. However, Study No. 4 (Hemchand et al., 2018), in which an appropriate

concentration of the stress factor was used, revealed lower degradation. A comparison of the results of hydrolytic studies shows that the duration of the stress test is influential on the extent of hydrolysis. Temperature also has an impact on the rate of hydrolysis. In Studies No. 9 and 10 (Harshalatha et al., 2018; Zaman & Hassan, 2021), the same concentration of acid and alkali and duration used were the same. However, Study No. 9 (Zaman & Hassan, 2021) was conducted at room temperature and Study No. 10 (Harshalatha et al., 2018) at 85°C.

In a comparison of the results of these two studies, the effect of temperature is evident, and greater degradation was observed for both drugs under acidic and alkaline conditions. Greater degradation was observed for VEL under higher temperatures. As previously mentioned, using temperatures higher than ambient conditions for oxidative stress tests may result in the formation of hydroxyl radicals, which are known to be very corrosive. Also, according to the guidelines, H_2O_2 concentrations are usually considered appropriate to range between 0.3-3% (Baertschi et al., 2016). Therefore, studies in which high concentrations of H_2O_2 are used or where the temperature is higher than room temperature are likely to produce unreliable results. What is critical is that sufficient exposure time should be considered for oxidative degradation to ensure the desired level of degradation is attained. Only in Study No. 9 (Zaman & Hassan, 2021) was an appropriate concentration of H_2O_2 used for the experiment conducted at room temperature for 7 days with an outcome that revealed that VEL was more sensitive to oxidative stress conditions than SOF. Applying thermal stress conditions revealed that both drugs are not sensitive to thermolytic conditions, and the duration of exposure is critical to causing stress. Therefore, sufficient time should be permitted to ensure thermolytic stress can be achieved. According to the guidelines, API thermal stress studies should be performed in the solid state at high under low humidity conditions (Baertschi et al., 2016). Only five studies used API in the solid state, and the tests were undertaken without humidity control. Photolytic stress testing revealed that both drugs were not very sensitive to the conditions.

Analysis of SOF and DAC

Two stability-indicating methods for the analysis of SOF and DAC in which stress tests were performed have been reported (Bandla & Ganapaty, 2018; Ramreddy Godela & Sowjanya, 2021).

Study No. 1, reported in (Table 4.) (Bandla & Ganapaty, 2018), used a high concentration of acid, base and H_2O_2. The stress testing results revealed that under acidic and basic hydrolysis in Study No. 2 (Ramreddy Godela & Sowjanya, 2021), more extensive degradation of both drugs drugs occurred. However, SOF was more sensitive to hydrolytic conditions than DAC. In study No. 2 (Ramreddy Godela & Sowjanya, 2021), a 3% v/v H_2O_2 solution at a temperature of 70°C was used for oxidative studies. As previously mentioned, oxidative testing with H_2O_2 is better if performed at ambient temperatures to avoid the possible formation of hydroxyl radicals. Both drugs exhibited limited degradation under thermal and photolytic conditions. However, thermal testing in this study was performed on a solution with humidity control. According to the guidelines, it is suggested that thermal testing be performed on API in the solid state at low and high humidity conditions (Baertschi et al., 2016).

Analysis of SOF, VEL and VOX

Stability indicating analytical methods for the analysis of SOF, VEL and VOX simultaneously with stress testing was reported in 5 studies (Balaswami et al., 2018; Deepthi & Sankar, 2020; Kokkirala & Suryakala, 2020; Lakshmi Maneka S et al., 2020; Padmini M et al., 2019).

Acid and alkali hydrolysis were performed with a high concentration of acid and alkali, and in all but one case, a temperature of 60°C was applied for 30 min. Neutral hydrolysis was also undertaken, and little degradation was observed. In all five studies (Balaswami et al., 2018; Deepthi & Sankar, 2020;

Kokkirala & Suryakala, 2020; Lakshmi Maneka S et al., 2020; Padmini M et al., 2019), oxidative stress was undertaken with 20% v/v H_2O_2 at a temperature of 60°C in studies No. 2 to 5 (Balaswami et al., 2018; Kokkirala & Suryakala, 2020; Lakshmi Maneka S et al., 2020; Padmini M et al., 2019), whereas in Study No.1 (Deepthi & Sankar, 2020) the temperature used was not reported and the percentage of degradation in all three drugs was almost the same. As mentioned before, it is better to have a H_2O_2 concentration of 3-6% and to do it at room temperature so that the DPs obtained are reliable. The thermolytic and photolytic testing conditions in these studies were similar and the results are in the same range.

Analysis of SOF and LED

The studies in which stability-indicating methods of analysis for SOF and LED are reported totals 15 (Bhavani & Maduri, 2020; El-Waey et al., 2023; El-Yazbi et al., 2020; Hassouna et al., 2017; Jahnavi & Ganapaty, 2018; Kumar & Rao, 2018; Kumari & Sankar, 2019; Mankar et al., 2019; Mastanamma et al., 2018; Narla & Pappula, 2020; Rao et al., 2017; Reddy et al., 2018; Rote et al., 2017; Suganthi et al., 2019; Veereswara Rao et al., 2018; Yeram et al., 2019).

Acid hydrolysis test conditions were considered suitable in Studies No. 13, but the alkaline stress test conditions used were unsuitable (El-Waey et al., 2023; Suganthi et al., 2019). Oxidative stress conditions in Studies No. 1,2,3,9 and 13 (Hassouna et al., 2017; Rao et al., 2017; Rote et al., 2017; Suganthi et al., 2019; Yeram et al., 2019) are better than others because an appropriate concentration of H_2O_2 of 3-6% controlled at ambient temperature was used.

CONCLUSIONS

To analyze an API in the presence of impurities, it is necessary to design short- and long-term stability studies based on valid guidelines while developing a stability-indicating analytical method for that API before validation of that method. The conditions used for stability studies should be identified and selected according to valid guidelines so that the degradation data generated are reliable. This review reveals that diverse approaches for the analysis of SOF are used, and the resultant data are highly variable and scattered. One of the important features of the studies reviewed is that they use short-term stability tests only and that no long-term stability testing was undertaken or reported. Since SOF has not yet been included in a monograph in any official pharmacopoeia, more detailed studies must be conducted using valid guidelines to produce important data relating to the degradation of the API, and DPs should be separated using a stability-indicating method specifically designed for routine analysis of the drug. The shortcomings identified in the studies investigated include not identifying the main DP and not reporting the kinetics or mechanism of degradation of the drug.

Recommendations

A review of published analytical studies for SOF has revealed that additional research is needed with respect to the analysis of SOF to overcome the shortcomings identified in previous studies and ensure that an accurate and reliable analytical method is designed to identify the API and its impurities. Accordingly, we have designed a study in our laboratory to perform stress and accelerated tests on the API and final product of SOF in accordance with valid guidelines. We aim to identify important DP so that a definitive stability-indicating analytical method for this API can be developed and validated. The main objectives of our study are to identify the degradation pathways, kinetics of degradation and purification of the important DP of the parent API.

AUTHOR CONTRIBUTION STATEMENT

Writing (N, HK), Writing (NN), Supervision (AM), Review and Editing (RB, W)

The contribution of authors, N, HK and NN is equal.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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