ADVANCING THE MANAGEMENT OF ANTI-RETROVIRAL DRUGS THROUGH GREEN ANALYTICAL METHODS

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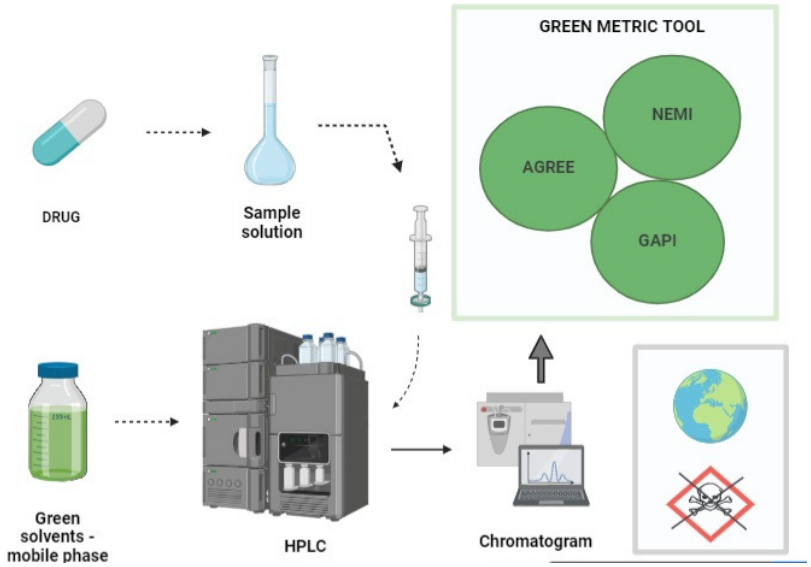
GRAPHICAL ABSTRACT:



Highlights:

1. Availability of green analytical method, shows the concern for the sustainability of environment.
2. Availability of different software for the measurement of Eco scale like -AGREE, GAPI, ECO-SCALE, NEMI.
3. Measurement of green score for different developed and validated ecofriendly analytical method like HPLC, UPLC, UV, Fluorimetry in different dosage form.

[1.]Foot Note, Green analytical chemistry aims to minimize environmental impact while maintaining analytical performances.

ABSTRACT

Conventional analytical techniques employ non-renewable organic solvents, which present major environmental risks and frequently produce large amounts of waste as a byproduct. Recent developments in green analytical techniques, such as the use of green solvents, the shrinking of analytical procedures, and the reduction of waste creation, are the main emphasis of the review. Using the various greenness scales, a range of analytical methods, including as potentiometry, spectro-fluorimetry, ultraviolet spectroscopy, high-performance liquid chromatography, and others, were assessed in accordance with the principles of green chemistry. Simple, free programs such as the Analytical Eco-scale, National Environmental Method Index (NEMI), Green Analytic Procedure Index (GAPI), and Analytical Greenness Calculator (AGREE) all attested to the fact that the data gathered using the suggested approach was environmentally acceptable and had no negative effects on the environment or human health.

KEY WORDS

Human immunodeficiency virus, Anti-retroviral drugs- zidovudine, lamivudine, Green analytical chemistry, Green solvents, Eco-friendly, Green metric tools-AGREE, GAPI, ECO-SCALE, NEMI.

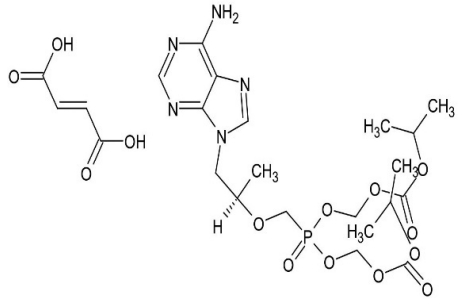
1.INTRODUCTION

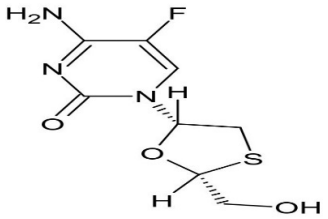
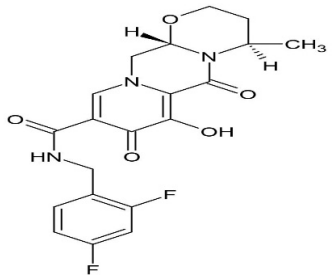
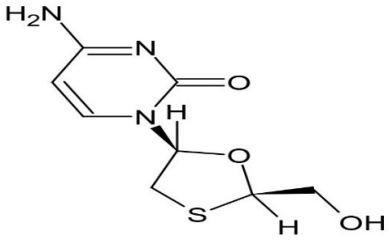
The pharmaceutical sector regularly uses dangerous and poisonous chemicals and solvents for quality testing, which puts the environment and the analyzers at serious risk. Beyond use, these concerns also apply to how these compounds are stored, transported, and disposed of. By lowering the number of required experimental steps, analytical chemistry can be a vital instrument in the advancement of green chemistry. Shorter analytical times, less energy use, and cheaper expenses result from this, supporting sustainable and ecologically friendly chemical research. Green analytical chemistry's main goal is to create or modify methods that reduce the usage of dangerous chemicals. Using environmentally friendly reagents and solvents, reducing chromatographic separation times, and downsizing analytical equipment are some ways to accomplish this [1–5]. Reducing the amount of chemicals used in reactions and substituting safer solvents for hazardous ones are important aspects of green analytical chemistry. Green chemistry's main idea is seen as a way to encourage sustainable development in analytical methods [6–10]. Analytical Eco-Scale and Green Analytical Procedure Index (GAPI) methodologies were used to evaluate the new method's efficacy and the environmental impact of the four existing methods.

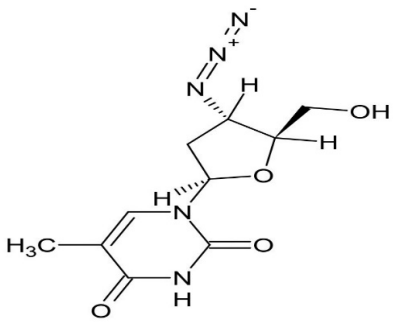
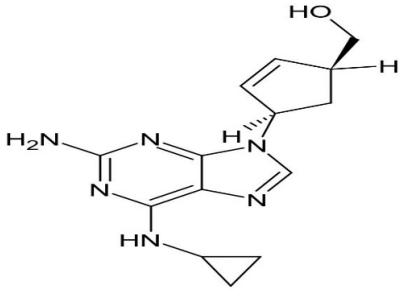
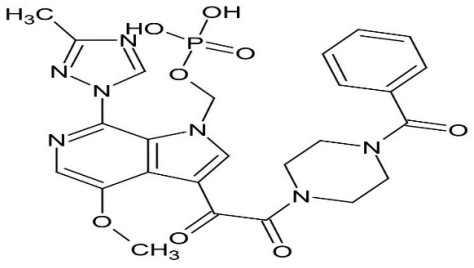
Acquired immunodeficiency syndrome, or HIV/AIDS, is still a serious global public health concern. It mostly affects groups with poorer viral suppression rates and higher risk, such as those infected through sexual contact and vertical transmission. Up to 52% of newborns born with HIV die before the age of two if treatment is not received, making young children and infants living with HIV particularly susceptible to negative outcomes [11–15]. Antiretroviral medications (ARVs) are essential for treating HIV infections because they prevent the virus from replicating, which slows the course of the illness and lowers the chance of transmission. In the past, chemotherapeutic agents such as Tenofovir disoproxil fumarate, emtricitabine, dolutegravir, efavirenz, ritonavir, lamivudine, zidovudine, abacavir, and fostemsavir have been used in various combinations and permutations as part of retroviral treatment in an effort to improve patient survival [16–19].

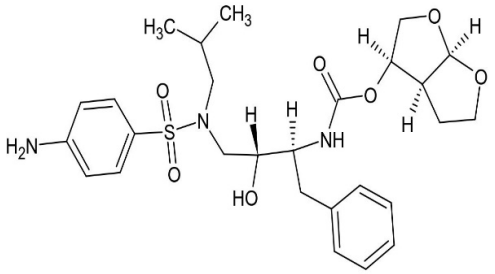
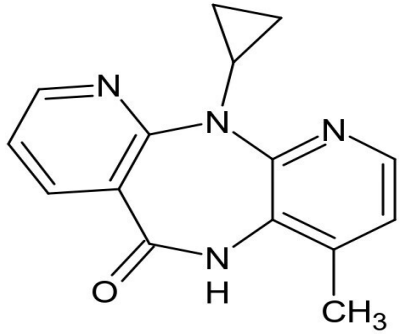
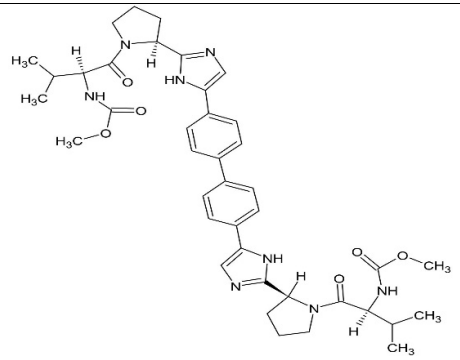
Anti-retroviral medications are evaluated using a variety of techniques, including spectrofluorimetry, UV, UPLC, and HPLC. Commonly used methods for detecting pollutants and degradation products in complicated mixtures or measuring several pharmacological medications are UPLC and HPLC. Several predetermination techniques and greenness testing methodologies for antiretroviral medications were found in the review [21–24].

1.1 Antiretroviral drugs analysed through green analytical methods.

Drug name	Physiochemical characters	Generic names	Method	Structures
Tenofovir disoproxil fumarate (TDF)	**Appearance:** irregularly-shaped crystalline powder **Solubility:** Soluble in water, methanol, and slightly soluble in ethanol. **Log P**: **1.25**, indicating moderate lipophilicity **MW**: 635.5g/mol **Melting Point:** 115–125°C.	Tenvir-EM, Ricovir-EM	HPLC, Potentiometric, UV, UPLC	

Drug name	Physiochemical characters	Generic names	Method	Structures
Emtricitabine	**Appearance**: White to off-white crystalline powder **MW**: 247.25g/mol **Solubility**: Freely soluble in water **Log P**: 0.43, indicating its hydrophilic nature **Melting Point**: 136-140 °C	Emtriva, Emcita	HPLC, UPLC	
Dolutegravir	**Appearance**: It is an off-white to pale yellow solid. **Solubility**: Slightly soluble in water, methanol and ethanol **Log P:** (2.5, indicating moderate lipophilicity **Melting Point**: 130-140°C. **Molecular Weight**: 419.38 g/mol	Dovato	HPLC, Spectro fluorimetry	
Lamivudine	**Appearance**: White or off-white crystalline powder **Solubility**: Freely soluble in water and ethanol **Log P**: -0.43 indicating it is hydrophilic. **Molecular Weight**: 229.26 g/mol **Melting Point**: 160-162°C	Zeffix, Lamivir-HBV, Epivir	HPLC, UV, Spectro fluorimetry	

Drug name	Physiochemical characters	Generic names	Method	Structures
Zidovudine	**Appearance**: Zidovudine is a white to off-white crystalline powder **Solubility**: Slightly soluble in water and Soluble in ethanol and methanol **log P**: -0.05, showing low lipophilicity. **Molecular Weight**: 267.24 g/mol **Melting Point**: 123-124°C	Viroz, Zidomax tablet and capsules	UV, HPLC	
Abacavir	**Appearance**: white to slightly yellow crystalline powder **Solubility**: It is soluble in water and methanol **Log P:** 1.04, indicating moderate lipophilicity. **Molecular Weight**: 286.33 g/mol **Melting Point**: 165-168°C	Ziagen	UV	
Fostemsavir	**Appearance**: White to off-white solid **Solubility**: slightly soluble in water and sparingly soluble in methanol, ethanol, and DMSO. **Log P**: Fostemsavir has a moderate partition coefficient **Molecular Weight**: 584.49 g/mol	Rukobia	UV	

Drug name	Physiochemical characters	Generic names	Method	Structures
Darunavir	**Appearance:** Darunavir is a white to off-white powder **Solubility:** It is poorly soluble in water but soluble in organic solvents **Log P**: 3.3, indicating moderate lipophilicity **Molecular Weight**: 547.66 g/mol **Melting Point**: 74–76°C	Prezista	HPLC, UV	
Nevirapine	**Appearance:** Nevirapine is a white to off-white crystalline powder **Solubility:** Slightly soluble in water and Soluble in methanol and ethanol. **Log P:** 2.5, indicating moderate lipophilicity. **Molecular Weight:** 266.3 g/mol **Melting Point:** 246°C to 250°C	Viramune	HPLC	
Daclatasvir	**Appearance**: Daclatasvir is a white to off-white crystalline powder. **Solubility**: It is slightly soluble in water, but freely soluble in ethanol, methanol **Log P:** The logP value is around 4.9, indicating its lipophilic nature **Molecular Weight**: 738.89 g/mol **Melting Point**: 160-170°C	Daklinza	UV	

2. ANALYTICAL METHODS

2.1 CHROMATOGRAPHIC METHODS

2.1.1 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Drug	Method	Sample	Chromatographic Conditions	Results	Reference
EMT	RP-HPLC	Capsules and oral solutions	**STATIONARY PHASE**: Nucleodur RP C18 (150mm×4.6mm,5µm) **MOBILE PHASE:** Methanol: ethanol (50:50%v/v) and flow rate of 1.5ml/min. **DETECTION**: UV at 280nm	Linearity-(0.999 ng/ml) Accuracy - (% recovery 94.00-109.00%) Precision Intraday-(3.041-5.16%) Interday- (3.19–4.93%) %RSD = 0.17–2.95 Low RT	[25]
LAM, ZDV	HPLC	Nanostructures	**STATIONARY PHASE**: Waters Atlantis, T3 (150mm×4.6mm,5µm) **MOBILE PHASE**: Citrate buffer: methanol (50:50%v/v) at pH6.5 and flow rate of 0.8ml/min. **DETECTION**: UV at 270 nm	Accuracy- 96.4% -96.6%,101.3% - 109.6% Linearity -21-39µg/ml, 42-78µg/ml r-0.994,0.994	[31]
TDF	RP -HPLC	Tablets	**STATIONARY PHASE**: SB C18 reversed phase column (150mm×4.6mm,5µm)	Linearity- 4.0-400.0ng/ml LOD-0.67ng/ml LOQ-2.2ng/ml	[28]

Drug	Method	Sample	Chromatographic Conditions	Results	Reference
			MOBILEPHASE: Methanol/water (60:40v/v) and flow rate of 1.0mL·min−1. **DETECTION**: UV at 260nm	Precision RSD-6.7% Recovery-91.4-99.6%	
3TC	HPLC	Tablets	**STATIONARY PHASE**: ARV4 C18 Column (250×3.0mm, 5µm) **MOBILE PHASE:** 0.1M ammonium acetate buffer and ethanol and flow rate of 0.4 mL/min. **DETECTION**: UV at 270nm	LOD (µg/ml)- 0.60, LOQ (µg/ml)-1.83, Accuracy%- 80%- 99.95 100%-100.07 120%-100.18 Intraday And Inter Day Precision: 80%-0.24,0.65 100%-0.32,0.36 120%-0.26,0.49 R^2-0.9994	[32]
ZDV	HPLC	Tablets	**STATIONARY PHASE:** C18column (ARV5µm250×3.0mm Interchim)	LOD- 1.18 ng/ml LOQ-3.58ng/ml Accuracy- 80%-100.14 100%-99.97	[32]

Drug	Method	Sample	Chromatographic Conditions	Results	Reference
			MOBILE PHASE:0.1M ammonium acetate buffer and ethanol and flow rate of 0.4ml/min. **DETECTION:** UV at 270nm	120%-100.25 Precision-Intraday and Inter day 80%-0.29,0.59. 100%-0.13,0.60 120%-0.29,0.64 R^2-0.9995	
NVP	HPLC	Tablets	**STATIONARYPHASE:** C18column (ARV5µm250×3.0mm, Interchim) **MOBILE PHASE**:0.1M ammonium acetate buffer and ethanol and flow rate of 0.4ml/min. **DETECTION:** UV at 270nm	LOD-2.04ng/ml LOQ-6.18ng/ml Accuracy- 80%-100.24 100%-100.00 120%-100.27 Precision Intraday and Interday 80%-0.43,0.17 100%-0.09,0.74 120%-0.64,0.75	[32]

Drug	Method	Sample	Chromatographic Conditions	Results	Reference
LAM, ZDV, NVP	HPLC	Powder form	**STATIONARY PHASE**: Phenomenex Octadecylsilane C-18 column (4.6 mm diameter × 0.25 m, 5 µm) **MOBILE PHASE:** 0.2M Ammonium acetate, methanol and acetonitrile (50:30:20%v/v) and flowrate of 0.6ml/min **DETECTION:** UV at 270nm	LOD – 3.80,7.03,4.64µg/ml LOQ -11.52, 21.29,14.18µg/ml Rt -5.5,7.8,11.1 mins Linearity-12.00-84.00µg/ml, 24.00-168.00µg/ml, and 16.00-112.00 µg /mL,	[33]

2.1.2. ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

Drug	Method	Sample	Chromatography Condition	Results	Reference
EMT	RP-UPLC	Tablets	**STATIONARY PHASE:** BEH C18 column (100mm×2.1,1.8µm) **MOBILE PHASE**: 0.68%Potassium dihydrogen ortho phosphate buffer and methanol (45:55v/v) Flow rate 1.2ml/min **DETECTION**: PDA detector 261nm	LOD -(ng/ml)-0.04,0.06 LOQ -(ng/ml)-0.15,0.225 Linearity-10-100ng/ml 15-150ng/ml R^2-0.998,0.999	[26]

Drug	Method	Sample	Chromatography Condition	Results	Reference
TFV	UPLC	Bulk powder	**STATIONARYPHASE:** Kinetex C18 column (50mm×2.1mm,1.7µm) **MOBILE PHASE:** Methanol and phosphate buffer (30:70v/v) and flow rate 0.2ml/min **DETECTION**: UV detection at 230nm	Linearity-1.0-2.0µg/ml RSD-below2% Accuracy-100.77%±0.11 RT-4mins	[29]
TAF	UPLC	Tablets	**STATIONARY PHASE**: Kinetex LC Column (30mm×2.1mm,1.7µm) **MOBILE PHASE**: 0.05M sodium dodecyl sulphate and 0.05Msodium dihydrogen Phosphate with 10%1-propanol (70:30v/v) and flow rate 1ml/min **DETETION:** UV at 210nm	Linearity- 1-18µg/ml RT-4.14mins , r-0.9996 Accuracy-100.77%±0.11 Precision-%RSD-0.30 LOD- 0.20µg/ml LOQ - 0.61µg/ml	[30]

2.2. POTENTIOMETRIC METHOD

Drug	Method	Sample	Reference Electrode	Results	Reference
TDF	Potentiometry	Tablets	Double-junction Ag/AgCl electrode. This was combined with the solid-contact ion-selective electrode (SC-ISE)	Linearity -0.01M-10µm LOD-7.5µm Nernstian slope-56.4mV/decade Accuracy-99.92%±0.90	[36]

				Precision- Intraday-1.45% Interday-1.92%	

2.3 SPECTROSCOPIC METHODS

2.3.1. FLUORIMETRIC METHOD

Drug	Method	Sample	Detection Wavelength	Results	Reference
DTG	Spectro fluorimetry	Tablets	Emission at 415nm and excitation at 262nm	Linearity -0.2-1.2ng/ml LOD-0.020ng/ml LOQ-0.061ng/ml Accuracy-99.2%-100.8%	[37]
LAM	Spectro fluorimetry using (GQDs)	Tablets	Emission at 435nm and excitation at 350nm	Accuracy-101.91% Precision Intraday-0.682% Interday-1.489%	[35]

2.3.2 UV SPECTROSCOPY

Drug	Sample Type	Method	Solvent	Detection Wavelength	Results	Reference
TDF	Bulk and dosage form	UV	Water,	(DW):235.5nm and 261.5nm (1D):274nm (RD):261.5nm and 252nm (1DR): 275.6nm	Linearity-5-35ng/ml (DW): LOD-0.144ng/ml LOQ:0.437ng/ml (1D): LOD:0.344ng/ml LOQ:1.04ng/ml (RD): LOD:0.916ng/ml LOQ-2.77ng/ml (1DR)-LOD-0.225ng/ml LOQ-0.684ng/ml Accuracy:(DW): 99.69±0.38% (1D)-99.40±0.21% (RD);99.77±0.38% (1DR):99.80±0.78%	[41]
ABV and LAM	Tablets	UV	HPLC-grade ethanol and distilled	228nm ,270nm	Linearity-1.5-3.0ng/ml 1.5-3.6ng/ml Accuracy-98%-102% RSD-below2%	[42]

Drug	Sample Type	Method	Solvent	Detection Wavelength	Results	Reference
			water as solvents			
FTR	Tablets	UV	Water	418 nm	LOD-0.10µg/ml LOQ-0.30µg/ml Linearity-2-12µg/ml R^2-0.9998 Accuracy-%R-99.08	[43]
DCV	Human plasma	UV	water miscible organic solvent	320nm	LOD-130ng/ml LOQ-400ng/ml R^2-0.9991	[44]
DTG and LAM	Tablets	UV	Ethanol Water	200-400nm	Linearity -1.5-32µg/ml, 1.5-36µg/ml LOD-0.330-0.358µg/ml, 0.473-0.478µg/ml Accuracy-99.61%,100.35% Precision-0.411%,0.347% R^2-0.9999	[34]

2.4 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY:

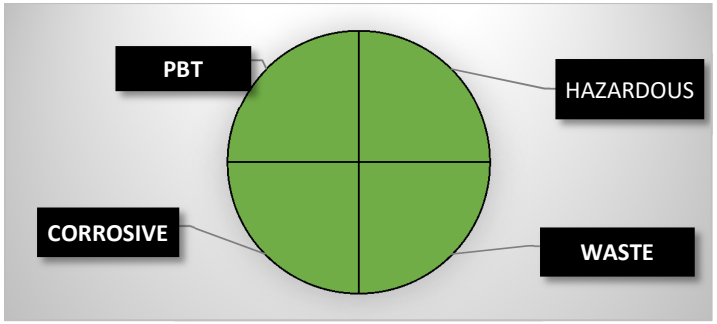
Drug	Sample Type	Solvent	Chromatographic Condition	Results	Reference
Tenofovir alafenamide	Tablets	n-butanol: acetic acid (7:3, v/v)	Stationary phase: HPTLC plates 20cm×20cm pre-coated with silica gel 60 F254 DETECTION: 260nm	Linearity-0.1-4µg/spot Correlation coefficient(r)-0.9997 Accuracy-98.95±1.09% Precision (RSD)-0.40% Intermediate precision (RSD)-0.37% LOD-0.02µg/spot LOQ-0.06µg/spot Rf-0.59 Rs-4.13 Tailing factor(T)-1 Recovery for alkaline degradation-98.98±1.23% For acidic degradation-97.95±0.56%	[30]

3. GREENESS ASSESSMENT OF ANTI-RETROVIRAL DRUGS:

The greenness of analytical methods is a critical factor in ensuring their sustainability By using innovative metric tools, the environmental impact of these methods can be analysed by the Greenness score, which helps in selecting and optimizing analytical procedures that minimize harm to the environment and human health. Numerous metrics have been introduced for evaluating the greenness of analytical methods such as NEMI, GAPI, Complex GAPI, Analytical Eco-scale, AGREE, providing valuable tools for assessing the greenness of analytical methods [45-54].

3.1. NEMI:(National environmental method index)

NEMI pictogram was created in 2002 by de la Guardia and Armenta NEMI pictogram has four parts If specific conditions have been satisfied, all the parts in the circle are filled with green, and the consumer is kindly referred to three criterion: The first criterion is that none of the chemicals is present on the persistent, bio accumulative and toxic chemicals list. The second criterion is that none of the chemicals applied in the procedure is listed on D, F, P or U hazardous wastes lists. The third criterion is that the pH of the sample is within 2–12 range to avoid a highly corrosive environment during the whole analytical process. The fourth criterion is must less than 50 g of waste is produced [55-59].



Example for NEMI Pictogram

3.2. ANALYTICAL ECO- SCALE:

Eco scale is a semi-quantitative tool to evaluate the greenness of an analytical method Any given reagent can be characterized with the help of the nine pictograms**,** which includes: flame, flame over circle, corrosion, gas cylinder, skull and crossbones, exclamation mark, environment, and health hazard. penalty points assigned for pictograms, signal words, waste generation, energy consumption, occupational hazardous, high-energy consumption, A Reagent without any pictograms receive 0 penalty point, if the signal word WARNING receives 1 penalty point, signal word DANGER receives 2 penalty points [46][55]. The analytical Eco scale is calculated by deducting the penalty points from base

score 100 the tool has a scale range from 0-100 where 0 represents a completely unsustainable reaction and high score represents the greener analytical method [60-67]

Eco scale = 100- Sum of Individuals Penalties

3.2.1 Penalty Points for Eco-Scale Score [60]:

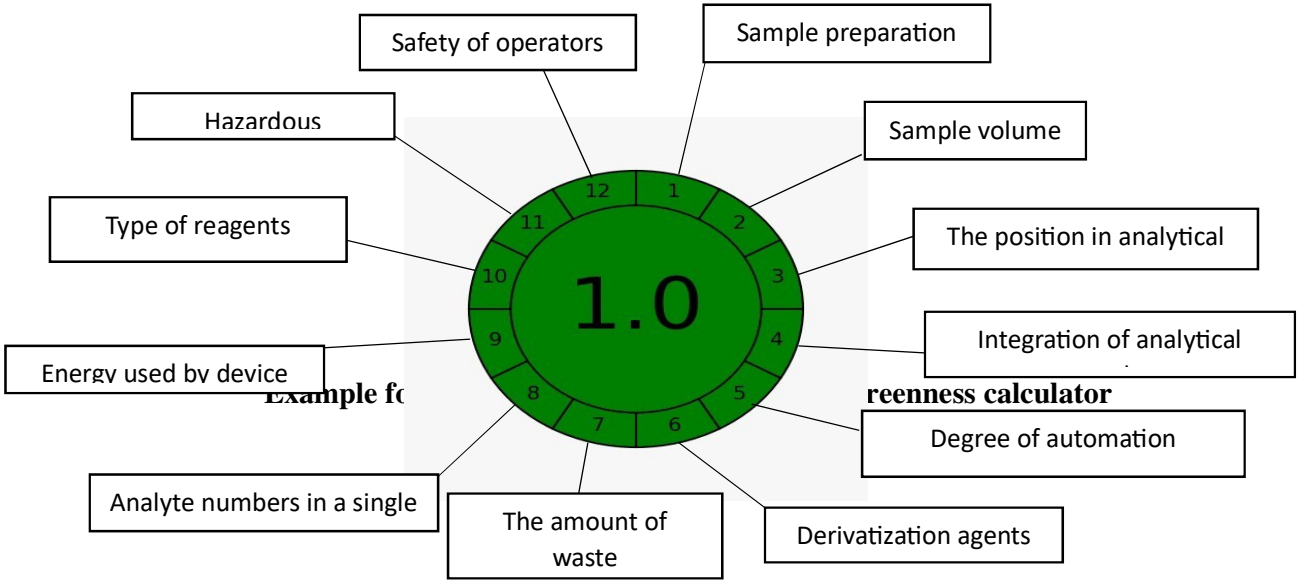
Reagents Used In Analytical Methods	Pictograms	Signal Word	Penalty Points
Chloroform	2	Danger	4
Methanol	3	Danger	6
Acetonitrile	2	Danger	4
Acetone	2	Danger	4
Benzene	3	Danger	6

3.3. AGREE: (ANALYTICAL GREENNESS METRIC APPROACH):

AGREE is a novel metric tool that evaluate the greenness of an analytical method according to the 12 GAC principles (SIGNIFICANCE) The pictogram is divided into12 sections corresponding to the 12 criteria [51][68]. The overall score is presented in the middle of the circle with values close to 1 and a dark green colour indicate that the analytical method is greener based on the performance of the GAC principle reflected by the colour in the segment, GAC principles 1,5,7 receive low scores, whereas principles 4,6,9 and 12 have excellent performance, Higher weights are given to principles 7,9 and a low weight is given to principles 1,5 and10. AGREE is an easily accessible software tool introduced by pena-pereira et al [69-73]. The AGREE open-access software can be obtained from https://mostwiedzy.pl/AGREE

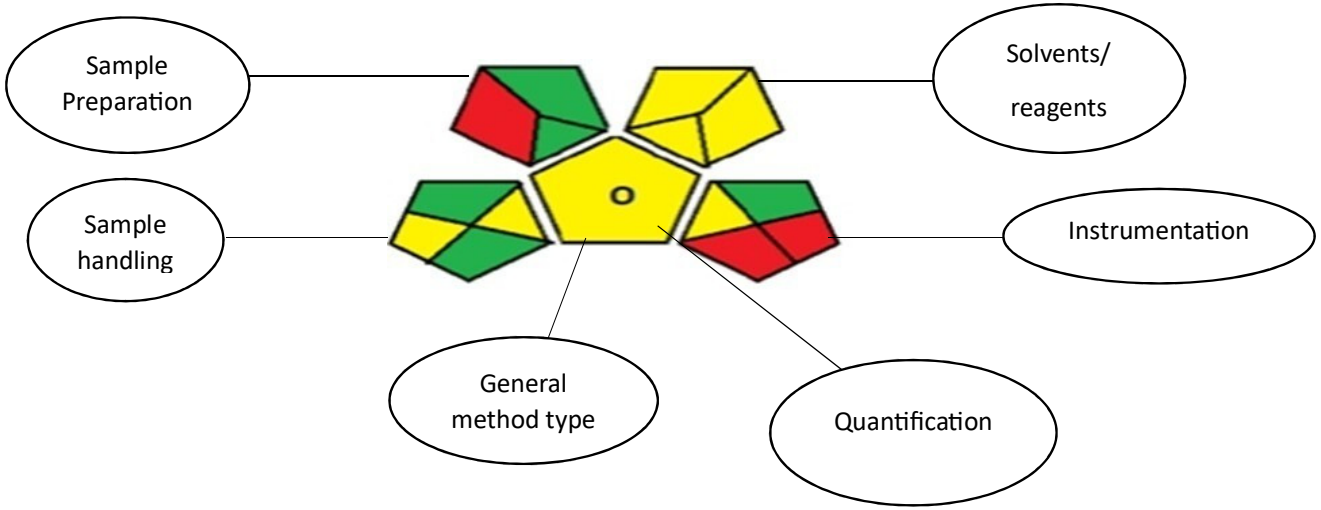
3.3.1. GAC (GREEN ANALYTICAL CHEMISTRY) PRINCIPLES

1. Direct analytical techniques should be applied to avoid sample treatment
2. Minimal sample size and minimal number of samples are goals
3. In situ measurements should be performed
4. Integration of analytical processes and operations saves energy and reduces the use of reagents
5. Automated and miniaturized methods should be selected
6. Derivatization should be avoided
7. Generation of a large volume of analytical waste should be avoided and proper management of analytical waste should be provided
8. Multi-analyte is preferred versus methods using one analyte at a time
9. The use of energy should be minimized
10. Reagents obtained from renewable source should be preferred
11. Toxic reagents should be eliminated or replaced
12. The safety of the operator should be increased



3.4. GAPI (Green Analytical Procedure Index):

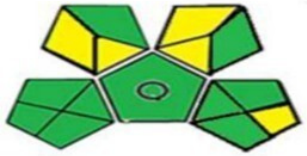
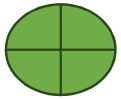
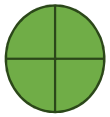
GAPI tool has five pentagrams to assess the greenness of an entire analytical method, from sample collection technique, sample preparation, transport, storage, conclusion, each part in the pentagram harmonizes with an analytical procedure, and the colour indicates greenness assessment, green, yellow and red colours used in the pentagram indicates low, medium, high environmental impact. The Complex GAPI tool, was created in 2021 features an extra hexagonal field at its base to enhance its greenness evaluation. Additional part corresponds to the greenness of pre-analysis process of the entire analytical procedure. GAPI tool used to evaluate green analytical procedures applied in the determination of anti-retroviral drugs.

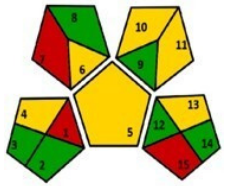


4. RESULT

4.1 GREENESS ASSESSMENT FOR ANTI-RETROVIRAL DRUGS

Drug	Approach	Greenness Score	Reference
FTR	UV	AGREE -0.82	[43]
TAF	UV	ECO SCALE -83 GAPI- 	[41]
LAM, ZDV, and NVP	RP -HPLC	AGREE-0.63	[33]
LAM, ZDV and NVP	HPLC	AGREE – 0.9 ECO SCALE – 75	[32]
TDF	POTENTIOMETRY	ECO-SCALE -94	[36]

TAF	UPLC and HPTLC	GAPI: UPLC-  HPTLC-  ECOSCALE: UPLC-89 HPTLC-85 NEMI: UPLC-  HPTLC- 	[30]
LAM and DTG	UV	ECO SCALE – 97 AGREE – 0. 79	[34]

EMT	RP-HPLC	AGREE – 0.72	[25]
ABC and LAM	UV	ECO SCALE – 97 AGREE – 0. 79	[42]
DCV	UV	ECO SCALE – 89 GAPI- 	[44]
LAM	Spectro fluorimetry	AGREE-0.75	[35]

5. CONCLUSION AND FUTURE ASPECTS:

In this review, we have emphasized the growing significance of adopting eco-friendly green analytical methods in the determination and monitoring of antiretroviral drugs. The review focuses on the simultaneous determination of multiple antiretroviral drugs using green chromatographic and spectrometric methods. The greenness of these methods was validated using the eco-scale protocol, proving their superiority over other reported methods. Additionally, the greenness of these methods was assessed using various tools such as the eco-scale assessment, NEMI, GAPI, and AGREE, with satisfactory results.

The future of green analytical methods for analysing anti-retroviral drugs appears optimistic, with remarkable development in chromatographic, spectroscopic techniques, and potentiometry methods, for future perspectives, many green metrics should be developed. By incorporating these metrics into a regulatory framework, industries and laboratories can more easily select and implement greener, more sustainable analytical

procedures. To facilitate this, the use of these metrics should be as easy as possible, where users can easily get the green rating. On the other hand, the creation of more metrics can lead to confusion and a lack of clarity. To avoid this, Harmonizing the different criteria into a single, easily applicable metric. These methods not only provide accurate and efficient analysis of anti-retroviral drugs but also correspond to green chemistry principles, reducing impact on the environment, minimizing health hazardous, being cost-effective, and promoting sustainability. The greenness of these methods has been demonstrated through several metrics such as the GAPI, Eco-Scale, and AGREE, NEMI. These results contribute to the pharmaceutical analysis field by providing a comprehensive understanding of the greenness for the analysis of these anti-retroviral drugs. Future studies could explore the application of these methods to analyse other anti-retroviral drugs or combinations of drugs by eco-friendly methods. These analytical methods along with green chemistry principles and green metric tools are essential for minimizing the ecological footprint of analytical chemistry and ensuring a sustainable future.

6. DATA AVAILABILITY STATEMENT:

Not available

7. ACKNOWLEDGEMENT:

This study is supported by the students and faculty of C.L.Baid Metha college of Pharmacy

8. CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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