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GREEN ANALYTICAL TECHNIQUES FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN WITH FENOFIBRATE AND EZETIMIBE: A COMPREHENSIVE REVIEW

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ABSTRACT

This article provides a critical evaluation of the analytical methods employed for the concurrent measurement of rosuvastatin calcium (ROS), fenofibrate (FEN), and ezetimibe (EZE) in pharmaceutical formulations and biological specimens. Focus is given to UV spectrophotometry, HPLC, and LC-MS/MS methods. ROS, a powerful HMG-CoA reductase inhibitor, is often administered in combination with FEN and EZE for managing high-risk individuals who fail to reach lipid targets with monotherapy. Several spectrophotometric techniques based on simultaneous equations, first-order derivatives, and various detection wavelengths have been described for ROS and FEN. Numerous RP-HPLC procedures using varying mobile phases, flow rates, and detection settings are available for ROS and FEN estimation. For ROS and EZE, methods involving HPLC, UPLC, and LC-MS/MS have been developed utilizing diverse columns, mobile phases, and detection systems. The review also introduces Green Analytical Chemistry (GAC) principles and evaluates the environmental sustainability of these methods using AGREE and GAPI tools. Emphasis is placed on the adoption of environmentally safer approaches without compromising analytical performance.

Keywords: Rosuvastatin, Fenofibrate, Ezetimibe, UV spectrophotometry, HPLC, LC–MS/MS, Green Analytical Chemistry, AGREE, GAPI

INTRODUCTION:

Rosuvastatin calcium (ROS) is a lipid-lowering drug that acts as a competing inhibitor of HMG-CoA reductase, the enzyme accountable for the rate-limiting step in cholesterol production – the conversion of HMG-CoA to mevalonate **[1]**. ROS is indicated for reducing blood levels of lipoprotein B, triglycerides, LDL cholesterol, and total cholesterol, while modestly raising HDL cholesterol levels. These actions are essential in decreasing the risk of atherosclerosis, a major contributor to cardiovascular complications such as stroke, peripheral vascular disease, and myocardial infarction **[2]**.

ROS is a synthetically derived compound and exists in the form of monocalcium salt of bis(C)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N methane sulfonyl aminopyrimidine)-5-yl)-(3R,5S)-dihydroxy-(E)-6-heptenoic acid **[3]**. The IUPAC name of fenofibrate is propan-2-yl 2-{4-[(4-chlorobenzoyl)phenoxy]-2-methylpropanoate}. Its main therapeutic objective is to lower elevated cholesterol levels in individuals at risk of cardiovascular events **[4]**. Ezetimibe (EZE), a member of the lipid-modifying class of drugs, is chemically known as 1-(4-fluorophenyl)-3(R)-[3-(fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. It functions by selectively blocking intestinal absorption of dietary cholesterol and plant sterols **[5]**. **Figures 1, 2, and 3** display the chemical structures of rosuvastatin, fenofibrate, and ezetimibe, respectively.

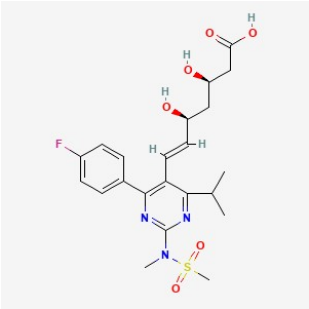


Figure 1: Structure of Rosuvastatin

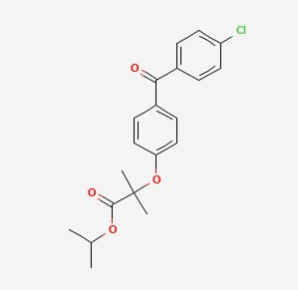


Figure 2: Structure of Fenofibrate

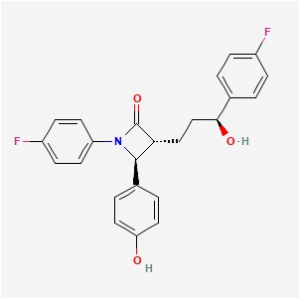


Figure 3: Structure of Ezetimibe

ROS is frequently combined with other lipid-lowering agents to improve outcomes in high-risk individuals who fail to achieve lipid targets with the maximum recommended dose of ROS alone **[6]**. Thus, combination therapies such as ROS with fenofibrate or ezetimibe are used. Clinical evidence shows a significantly higher percentage of patients achieving their NCEP ATP III cholesterol goals when treated with ROS and ezetimibe together compared to ROS monotherapy ($p < 0.001$) **[7]**. Several Analytical techniques are available to evaluate ROS as an individual drug and also in combination with other drugs. This review compiles various analytical procedures used to quantify Rosuvastatin, Fenofibrate, and Ezetimibe.

ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN WITH FENOFIBRATE AND EZEMITIBE:

Multiple UV spectrophotometric methods have been proposed for concurrently estimating ROS and FEN, as detailed below. R.R. Sevda *et al*. utilized a simultaneous equation method in a UV spectrophotometric technique, detecting wavelengths of 244 nm for Rosuvastatin and 286.7 nm for Fenofibrate **[8]**. B. Patel *et al*. devised a UV spectrophotometric technique with peak absorption wavelengths at 243 nm and 224 nm **[9]**.

Karunakaran *et al*. introduced a UV spectrophotometric method utilizing the simultaneous equation technique, with λmax values of 243 nm for ROS and 287 nm for FEN in methanol. Furthermore, they established an HPLC technique utilizing a Luna C18 column functioning in reverse-phase mode. The concentration ranges exhibiting an uniform relationship were 1–7 µg/mL for ROS and 4–28 µg/mL for FEN **[10]**. V. Parmar *et al*. devised a first-order derivative UV spectrophotometric technique to quantify FEN and ROS in tablet formulations **[11]**.

Several HPLC methods have been formulated for simultaneously quantifying Rosuvastatin and Fenofibrate.

Gaikwad *et al*. developed an RP-HPLC method utilizing a mobile phase composed of methanol and water (90:10 v/v), which was degassed through ultrasonication.

Detection occurred at 254 nm with a flow rate of 1.0 mL/min. The retention times were 3.316 minutes for ROS and 4.413 minutes for FEN **[12]**.

Sumalatha and Pavani utilized an alternative RP-HPLC method using a mobile phase composed of OPA buffer (pH 3.0) and methanol in a 65:35 v/v ratio, with a flow rate of 1.2 mL/min. Detection occurred at 238 nm. ROS and FEN exhibited retention durations of 1.950 and 3.858 minutes, respectively **[13]**.

Several analytical techniques such as HPLC, UPLC, and LC-MS/MS have also been established for the simultaneous estimation of Rosuvastatin and Ezetimibe.

Sri *et al*. employed an Enable C18G column (5 µm, 250 mm × 4.6 mm i.d.) in a study to separate two medicines, utilizing a mobile phase of acetonitrile and water (75:25 v/v) at a flow rate of 0.6 mL/min. Detection transpired at 252 nm **[14]**.

Gajjar and Shah developed a RP-HPLC method employing mobile phase composed of 0.05 M phosphate buffer (pH 2.5) and methanol in a 45:55 v/v ratio. Detection occurred at 242 nm at a flow rate of 1.0 mL/min **[15]**.

A separate RP-HPLC approach developed by Ashfaq *et al*. facilitated the concurrent quantification of Ezetimibe and Rosuvastatin in human plasma. Analytes were extracted utilizing acetonitrile and protein precipitation, succeeded by separation with a C18 column. A diode array detector quantified the eluent at 240 nm. The mobile phase comprised acetonitrile and 1.5% phosphoric acid at a ratio of 30:70 (v/v) **[16]**.

Mukthinuthalapati *et al*. devised a stability-indicating reversed-phase liquid chromatography method with a photodiode array detector and a C18 column. The mobile phase comprised sodium acetate buffer (pH 4.0) and acetonitrile (30:70 v/v), with a flow rate of 1.2 mL/min and detection at 254 nm **[17]**.

Varghese and Ravi validated a liquid chromatography/electrospray ionization mass spectrometry technique for measuring reactive oxygen species and ezetimibe in plasma with an isocratic mobile phase of 0.1% formic acid–methanol (20:80 v/v). Detection utilized selective ion monitoring at m/z 480 (ROS) and m/z 408 (EZE) **[18]**.

GREEN ANALYTICAL CHEMISTRY (GAC):

The concept of Green Analytical Chemistry (GAC) encourages analytical scientists to prioritize health, safety, and environmental considerations during method development **[19]**. Assessing the greenness of analytical techniques is complex due to the multifactorial nature of the parameters involved **[20]**. This review explores two prominent greenness evaluation tools: AGREE and GAPI. Since defining greenness is challenging, it is essential that

evaluation tools consider various factors. The relevance of greenness in analytical chemistry is effectively represented through the 12 principles of GAC **[21]**.

The Green Analytical Chemistry Principles:

1. Use direct analysis to avoid complex sample processing.
2. Minimize sample volume and reduce the number of samples analyzed.
3. Prefer on-site (in-situ) analysis techniques.
4. Combine analytical steps to reduce reagent use and energy.
5. Favor miniaturized and automated techniques.
6. Avoid chemical derivatization steps.
7. Limit the production of analytical waste and ensure its proper disposal.
8. Prefer multi-analyte over single-analyte procedures.
9. Decrease energy consumption in all stages.
10. Opt for reagents from renewable sources.
11. Substitute or eliminate hazardous chemicals.
12. Ensure maximum safety for laboratory personnel

Each of the twelve GAC principles has been translated into a scoring metric for a more complete evaluation of analytical method greenness

AGREE (Analytical GREENness metric):

It is the metric system that utilizes the 12 principles of GAC to represent the greenness in a clock like pictogram that is coloured and present the score at the middle. It also provides a report that states the individual score for every principle and differentiate them in different colours which includes red, yellow and green. **Figure 3** represents the model AGREE pictogram **[21]**.

Figure 4: Represents AGREEmetric GREENness score

GAPI (Green Analytical Procedure Index):

The Green Analytical Procedure Index (GAPI) is a semi-quantitative assessment instrument that evaluates the environmental impact of analytical operations from sample collection to final analysis. GAPI, developed by Nowak and Koel in 2018, evaluates multiple factors, including solvents, instruments, waste, and sample

preparation. To represent the environmental impact of each stage, the GAPI tool use a color-coded pictogram that is red, yellow, and green. This visual style enables swift comparison of methods and aids in recognizing more sustainable options **[22]**. Unlike the older NEMI label, which assessed only four features, GAPI delivers a more comprehensive profile. Its application supports the development and selection of more sustainable and resource-efficient analytical strategies **[23]**.

RESULTS:

In this review, GAPI and AGREE tools were applied to the reported methods, and the resulting pictograms illustrate their respective environmental impact. A summary of the greenness evaluations is presented in the following **Table 1**.

Table 1: Spectrophotometric methods for the analysis of ROS and FEN simultaneous from dosage form.

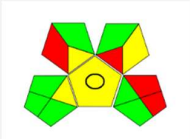
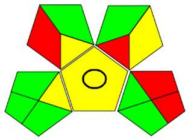
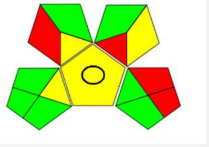
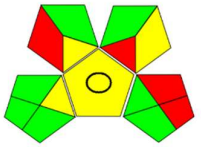
ANALTYE	MATRICES	WAVELENGTH	SOVENT / REAGENT	LINEARITY RANGE	AGREE	GAPI	REFERENCE
ROS; FEN	Bulk and tablets	244ROS;286.7FEN	MeOH	1–10µg/mL			[1]
ROS; FEN	Tablets	243ROS;224FEN	MeOH	4–12 µg/mL			[2]
ROS; FEN	Tablets	243ROS;287FEN	MeOH	1–7 µg/mL			[3]
ROS; FEN	Tablets	224FEN;243258ROS	MeOH	4–12 µg/mL			[4]

Table 2: Reported analytical HPLC and UPLC methods for the determination of ROS in combination with fenofibrate in pharmaceutical dosage forms and biological matrices

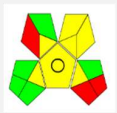
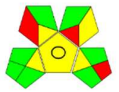
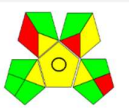
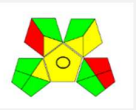
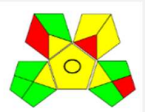
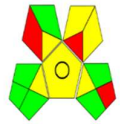
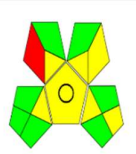
Analyte(s)	Matrices	Detection wavelength	Mobile phase	Stationary phase	Flowrate (mL/min)	Retention time (min)	Linear range for ROS	AGREE	GAPI	Reference
ROS; FEN	TAB	252	ACN:MeOH:water (50:40:10, v/v)	Luna C18 column (250£4.6mm, 5mm	0.5	2.60	1–7 µg/mL			[3]
ROS; FEN	Bulk and tab	254	MeOH: water (90:10v/v)	Zodiac C18 column (250£4.6mm)	1	3.316	10–50 µg/mL			[5]
ROS; FEN	Tab	238	Phosphoricacid (pH3.0): MeOH (65:35v/v)	Hypersil C18 column (250£4.6mm, 6.5mm)	1.2	3.858	50–150 µg/mL			[6]

Table 3: Reported analytical HPLC, UPLC and LC-MS/MS methods for the determination of ROS in combination with EZE in pharmaceutical dosage forms and biological matrices

Analyte (s)	Matrices	Detection wavelength	Mobile phase	Stationary phase	Flowrate (mL/min)	Retention time (min)	Linear range for ROS	AGREE	GAPI	Reference
ROS; EZE	TAB	252	ACN: water (75:25, v/v)	Enable C18G column (250£4.6mm, 5mm)	0.6	2.931	5–40 µg/mL			[23]
ROS; EZE	TAB	242	0.05Mphosphate buffer(pH2.5): MeOH(45:55v/v)	HypersilC18 column (150£4.6mm, 5mm)	1	5.55	5–80 µg/mL			[7]
ROS; EZE	Human plasma	240	1.5%phosphoric acid: ACN (30:70v/v)	MerckC18column (250£4.6mm, 5mm)	1	3.358	0.32–267 µg/mL			[8]
ROS; EZE	TAB	254	Tetrabutyl ammonium hydrogen sulphate: ACN (32:68v/v)	C18column(250£4.6mm,5mm)	1	3.54	0.1–200 µg/mL			[9]
ROS; EZE	Huma plasma	LC–MS/MS ESI- SIM480	0.1%(v/v) formic acid: MeOH (20:80v/v)	LunaC18column (150£4.60mm, 5mm)	1	2.7	0.1–10 µg/mL			[10]

CONCLUSION:

The numerous analytical methods created for the simultaneous measurement of Rosuvastatin (ROS) in combination with Fenofibrate (FEN) and Ezetimibe (EZE) in pharmaceutical formulations and biological matrices are critically examined in this extensive review. UV spectrophotometry, RP-HPLC, UPLC, and LC–MS/MS are among the techniques that have been discussed; each has unique benefits with regard to sensitivity, specificity, and applicability. The review also highlights how crucial it is to implement Green Analytical Chemistry (GAC) principles in order to reduce the environmental impact of these techniques. Analytical processes have been effectively evaluated for greenness using tools like AGREE and GAPI, which offer a measurable and visual illustration of their sustainability.

Additionally, a number of techniques showed encouraging green scores, suggesting a shift and increased awareness of ecologically friendly analytical techniques. Pharmaceutical analysis has advanced substantially with the incorporation of greenness assessment and analytical performance. To enable wider adoption, additional effort is required to standardize green metrics among labs and regulatory agencies.

Miniaturization, waste reduction, energy efficiency, and safer reagents should remain top priorities in the development of analytical methods in the future—all without sacrificing accuracy or robustness. In the end, the pharmaceutical industry's dedication to quality, safety, and environmentally responsible innovation will be strengthened by the convergence of analytical efficiency and environmental sustainability.

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