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A Green Raman Spectroscopic Assay Method for The Quantification of Tranexamic Acid in Pharmaceutical Formulations

P. S. Singh[1], R.S. Lokhande[1] and D. A. Kochrekar[2]

[1]School of Basic Sciences, Department of Chemistry, Jaipur National University, India;
[2]FDC Limited, Mumbai, Maharashtra, India

E-mail/Orcid Id:

PS, prysng21@gmail.com, https://orcid.org/0000-0003-3480-5504; *RL,* rama.lokhande@yahoo.com, https://orcid.org/0000-0002-6006-4757; *DK,* dkochrekar01@gmail.com, https://orcid.org/0009-0007-2400-8903

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Abstract: Tranexamic acid (TXA) is a widely used antifibrinolytic agent that is used to prevent and treat excessive bleeding. Current analytical methods for TXA are often time-consuming and require the use of toxic solvents. Raman spectroscopy has the ability to measure TXA because it is a fast and non-destructive analytical method. This research established and validated a green Raman spectroscopic test for TXA measurement. There was no need for harmful solvents because the technique relied on a basic aqueous solution of TXA. Results demonstrated that the technique was linear across the 0.7% to 13.0% concentration range. The method was also shown to be accurate, with results comparable to the orthogonal techniques. The green Raman spectroscopic assay method was applied to the quantification of TXA in pharmaceutical formulations. The results obtained were comparable to those obtained using reference methods. To quantify TXA quickly and accurately, one can use the green Raman spectroscopic test. Since no harmful chemicals are needed for the procedure, it is also eco-friendly. Method has the potential to be used in a variety of settings, including pharmaceutical quality control and research laboratories.

Introduction

The medicine tranexamic acid (TXA) is used to prevent and treat excessive bleeding. It is an artificial version of the protein lysine. TXA works by blocking the action of plasmin, a protease that breaks down blood clots. This helps to keep blood clots from dissolving and reduces the risk of bleeding.

In the current scenario, most pharmaceutical production laboratories use different technologies to determine content in different drug dosage forms. These techniques include Chromatography, UV spectroscopy, IR spectroscopy, Electrophoresis, Immunoassay etc. Among all these, the most common method employed in quality control laboratories involves High-performance liquid chromatography for Assay and content uniformity determination. Although this method is sensitive enough, it has many disadvantages since it is destructive and includes hazardous chemicals, consumables, energy consumption, and long run times. With the awareness brought in through the introduction of green analytical chemistry to develop and promote the use of eco-friendly analytical methods, scientists have developed and introduced environmentally safe techniques that reduce environmental hazards. Recent advances in technologies have emerged over the past few years, including the new analytical tool Transmission Raman Spectroscopy (TRS). This research paper is more focused on utilizing the capabilities of TRS to develop an analytical method for

***Corresponding Author:** prysng21@gmail.com

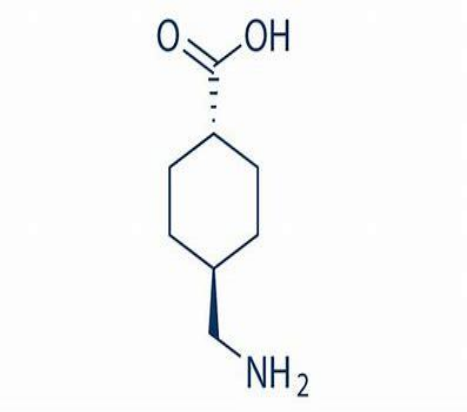

Figure 1. Structure of Tranexamic acid

determining the content of TXA in the injection formulation. Several methods are available for the quantification of TXA by employing different analytical techniques, including chromatographic methods such as HPLC and GC, that are widely used to determine TXA in pharmaceutical formulations and biological samples. These methods are highly sensitive and selective but can be time-consuming and expensive to perform (Patil et al., 2017). Spectrophotometric assay methods are developed using derivatization agents since TXA does not have a chromophore group. These derivatisation agents make the method less green (Gadkariem et al., 2012). TXA in marketed samples has been tested using the Zero-Order Infrared Spectrophotometry methods. These methods are validated and used in routine analysis. These methods are also found to be green in nature (Nerdy et al., 2021; Sparén et al., 2015; Pelletier et al., 2012). Electrophoretic methods such as capillary electrophoresis (CE) can also be used to quantify TXA. These methods are relatively simple and inexpensive to perform, but they may not be as sensitive or selective as chromatographic methods (Wang, 2020; Ciurba et al., 2013). Immunoassay methods such as enzyme-linked immunosorbent assay (ELISA) can be used to quantify TXA in biological samples. These methods are highly sensitive and selective, but they can be time-consuming and expensive to perform (Gleeson et al., 1994).

TRS is a non-destructive analytical technique that can be used for the quantification of TXA in pharmaceutical formulations and biological samples (Eliasson et al., 2014; Shimamura et al., 2019). This method is quick and simple to perform, and it does not require the use of any toxic solvents (Griffen et al., 2015). The assay for Tranexamic acid using the Raman spectroscopy has not been explored (Buckley and Matousek, 2011). The current research will focus oncapacity of TRS to quantify Assay of TXA in pharmaceutical formulations (Li et al., 2016; Andrews et al., 2018; Niedziałkowski et al., 2019).

The search for environmentally friendly ways to measure TXA has gained momentum in recent years (Everall et al., 2010). Green analytical methods are those that are ecologically friendly and minimize use of toxic solvents. One example of a green analytical method for quantification of TXA is Raman spectroscopic method mentioned above (Franson et al., 2010; Anastas, 1998).

The choice of an assay method for the quantification of TXA will depend on a number of factors, including the type of sample being analyzed, the required sensitivity and selectivity, and the available resources (Silge et al., 2022).

Raman Spectroscopy technique

The Raman spectroscopy method examines the vibrational and rotational modes of molecules through the non-destructive analysis of light scattering. When a photon of light strikes a molecule, it can be absorbed and re-emitted with a different energy. This energy alteration relates to vibrational or rotational energy of molecules (Orlando et al., 2021). By measuring the energy of the scattered photons, TRS can be used to recognize molecules present in a sample and to study their structure and dynamics (Johansson et al., 2007)

One of the many applications of Raman spectroscopy is the analysis of biological samples, in addition to solids, liquids, gases, and more. It is also a very sensitive technique and can be used to detect trace amounts of materials (Sha et al., 2023). As compared to other analytical methods like X-ray diffraction and infrared spectroscopy, Raman spectroscopy offers certain benefits. It can assess samples in a variety of conditions, including in situ, and is non-destructive. Additionally, it does not require sample preparation (Everall et al., 2010; Liu et al., 2019; Liu et al., 2020; Shi et al., 2019).

TRS is used in a wide variety of fields, including Materials science to study the structure and properties of materials such as semiconductors, ceramics, polymers, and composites. Proteins, nucleic acids, and lipids are just a few examples of the biological molecules that it helps researchers understand better (Omar et al., 2020). In the field of Chemistry, it is used to study the composition and structure of chemicals, including pharmaceuticals, explosives, and environmental pollutants. Diagnosis of diseases and to guide treatment in field of medicine is conducted. Raman spectroscopy is used to identify materials and to determine their origin (Inoue et al., 2019).

The goal of this study was to create a technique for analyzing the content of TXA injections by combining transmission Raman spectroscopy with PLS (Steinbach et

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al., 2017). To forecast the amount of TXA in the injection samples, model was improved by alteringwavelength,signal collector, preprocessing technique, and other factors. HPLC method was subsequently used to correct model (Zhao et al., 2022). We estimated and quantified the API contents of commercially available TXA injectable samples, and then compared those results with those from the HPLC analysis. The similarities between the two cases indicate that the model could be useful for pharmacological examination of final goods (Andrews et al., 2018; Villaumié et al., 2018). The process for model building is shown in the Figure 2.

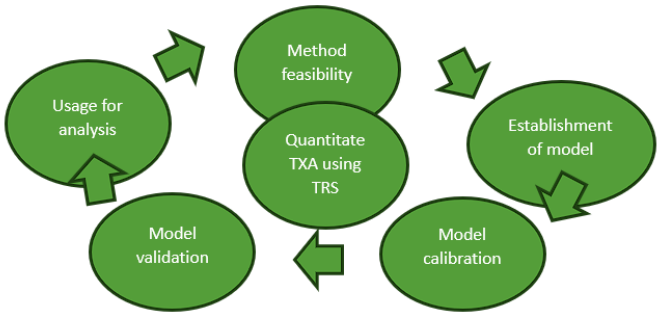

Figure 2. Process of model building

Materials and Methods

The analysis was carried out QT Raman instrument with a Raman probe from Metrohm. Samples of TXA injection from MacLeod's pharma were used for the study.Water was used as the diluent. The drug substance of TXA was used for building the calibration model. The solutions were prepared and the solution measurements were performed on TRS instrument.

Figure 3. QTRam instrument used for Raman Spectroscopy analysis

Measurement of Raman Spectra

A glass vial was used to collect TXA injection samples, and a QT Ram Raman spectrometer (BWT-840000893) equipped with a Raman probe (BAC 102-785-ST) was used to obtain Raman spectra. The QT sampler used was RSA001. The QTRam optics and sample holder were set at 4mm. The measurement was conducted using a laser operating at 785 nm wavelength. The laser power was kept at maximum (100%) and the integration was done in 5-second time intervals. The glass vials used for measurement were of 15 × 26 mm capacity (Matousek et al., 2011).

Results and Discussion

The Raman spectra obtained for TXA injection and TXA powder are presented in Figure1 and Figure 2. Solution of TXA powder (API) is prepared at 10% concentration to be in line with the concentration of TXA in the injection sample. The solutions of TXA prepared from the sample and API are taken in glass vials and the measurement is performed. A clear spectrum was obtained with distinct peaks for TXA from 250 to 1550 cm^{-1}.

Different synthetic samples were prepared for building the calibration model by using TXA injection, API, and ultrapure water (Lim et al., 2018). The levels included solutions from 7.0% to 13.0%. The Raman spectra obtained for the calibration levels are presented in Figure 4. Each level represented a clear spectrum with distinct peaks in the region selected for measurement. A second derivative of the synthetic samples was also plotted for use in building the calibration model (Figure 5).

Calibration spectra from each level between 7.0% to 13.0% were used to build predictive models for the constituent TXA under evaluation. The results indicate that PLS model is built with acceptable model parameters for TXA.

Theoretical values of commercial samples and synthetic samples were used for model creation. Linearity was checked with different concentration level ranging from 7.0% to 13.0%. Spectral response was found to be linear with respect to concentration. Correlation value was found to be above 0.9999.

From the PLS model, it can be observed that the TXA exhibits good correlation with value of 0.9999 at Press factor 4.

Table 1. Parameters for model building

Sample	Tranexamic acid Injection TXA
Treatment	First Derivative Seg $ Gap 10
Factor	4
Correlation	0.9999
Wavelength region	250 – 1550 cm^{-1}

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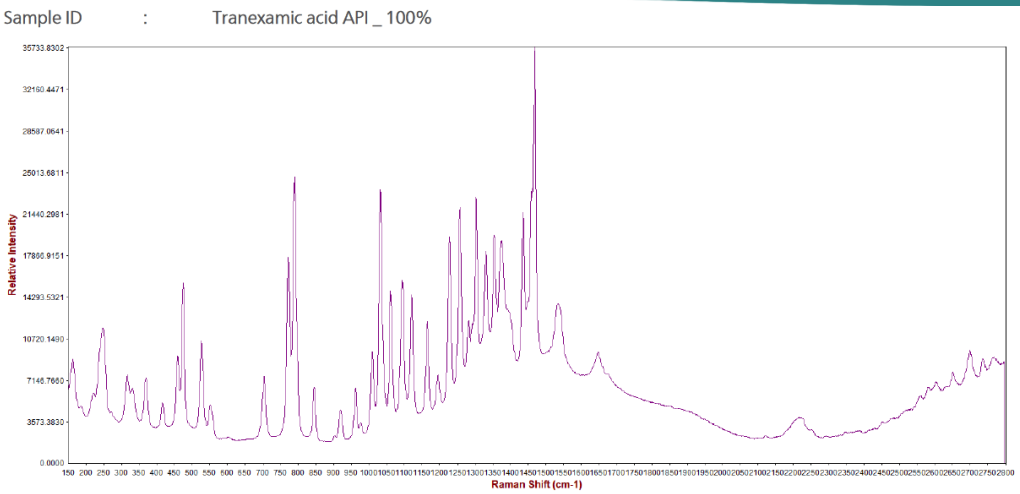

Figure 4. Raman Scan for API at 100% concentration

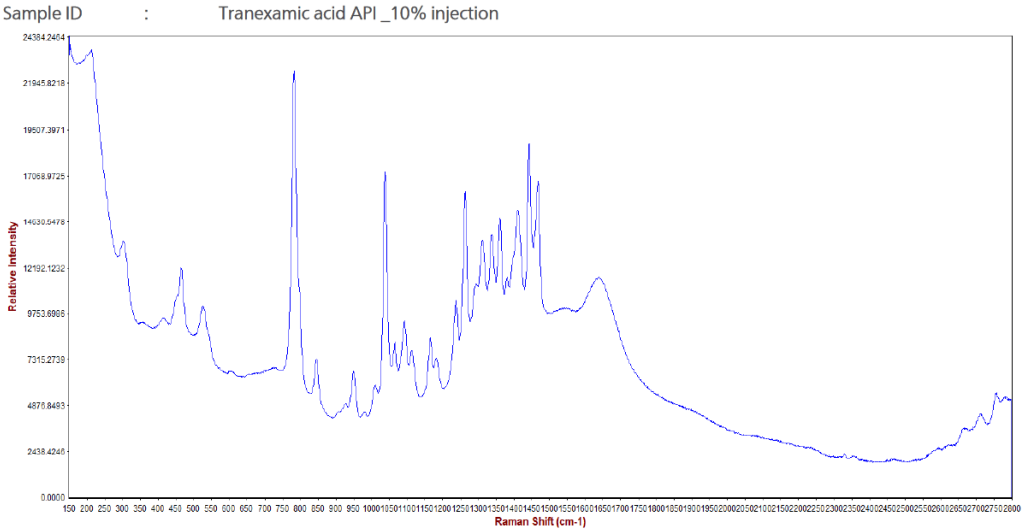

Figure 5. Raman Scan for Sample solution

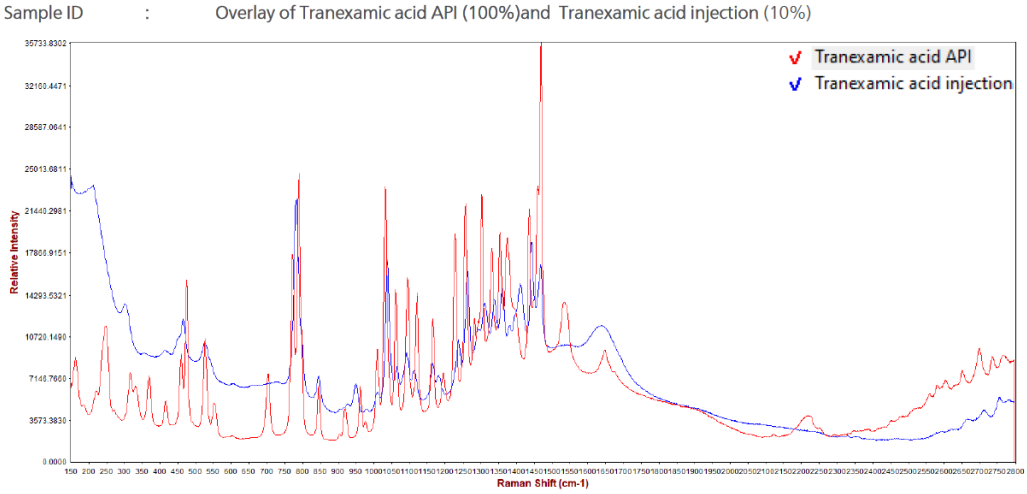

Figure 6. Overlay scan of API and Sample solution showing matching bands

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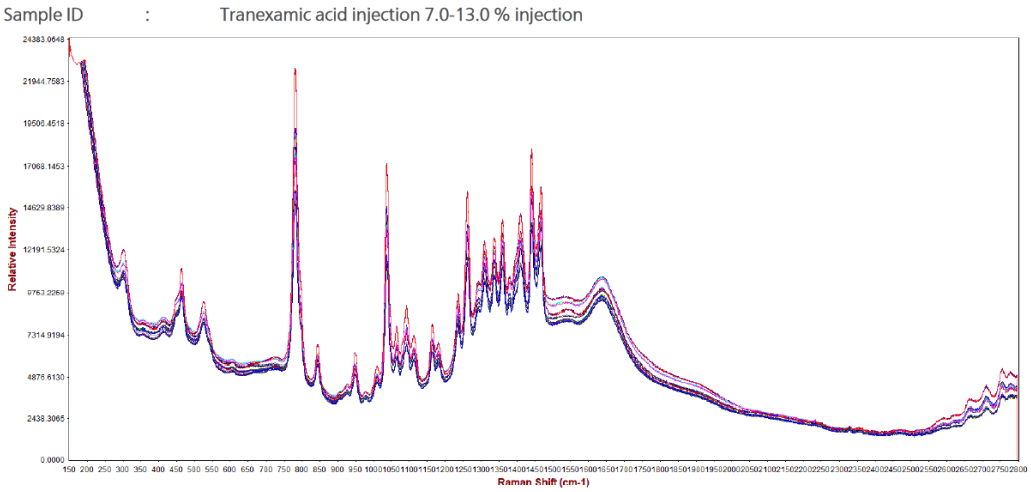

Figure 7. Overlay Raman scan of linearity levels from 7.0% to 13.0% levels

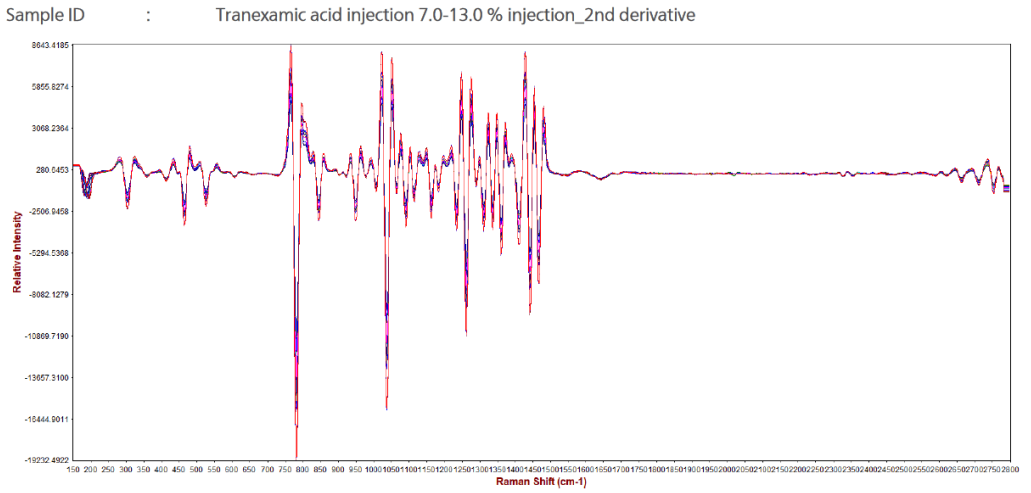

Figure 8. Overlay Raman scan of second derivative for linearity levels from 7.0% to 13.0% levels

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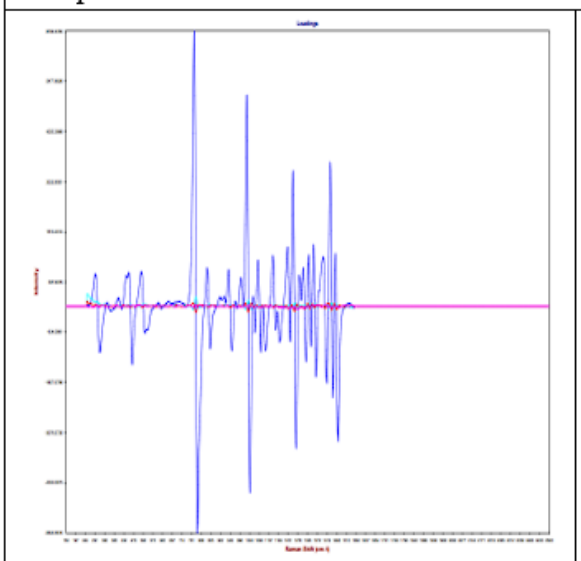
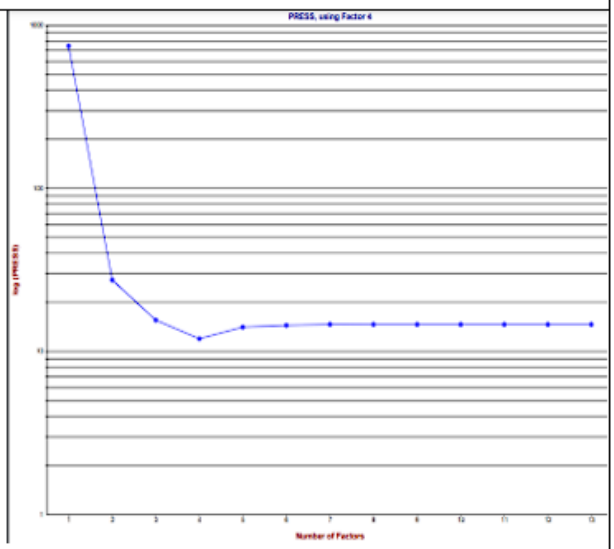
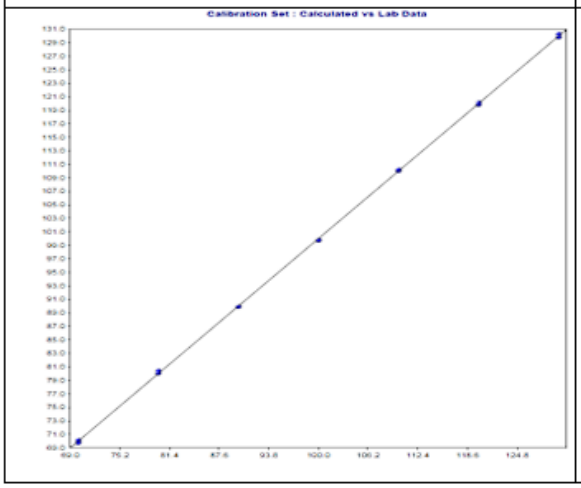
Sample ID: Calibration model for TXA 7% to 13%







Factor	R squared	Std Error calib	PRESS	F-value	Std Error X validation
Factor 1	0.922539	5.851996	745.8682	226.2851	5.959659
Factor 2	0.997777	1.018647	27.39678	4038.688	1.142195
Factor 3	0.999568	0.461916	15.56837	13116.61	0.861017
Factor 4 *	0.999909	0.218961	11.95622	43800.74	0.754549
Factor 5	0.999943	0.17868	14.08949	52590.15	0.819102
Factor 6	0.999996	0.047425	14.46516	621376.1	0.82995
Factor 7	0.999999	0.018707	14.64846	3461964	0.835192
Factor 8	1.0	0.006757	14.63433	25165822	0.834789
Factor 9	1.0	0.001334	14.63809	1.8E+308	0.834896
Factor 10	1.0	0.000389	14.63909	1.8E+308	0.834925
Factor 11	1.0	6.73E-05	14.63929	1.8E+308	0.83493
Factor 12	1.0	2.44E-05	14.63916	1.8E+308	0.834927
Factor 13	1.0	8.39E-06	14.63916	1.8E+308	0.834927

Figure 9. Calibration Model for Tranexamic acid injection (TXA)

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The commercial samples of TXA manufactured by MacLeod's Pharmaceuticals were measured using the QTRam instrument and the calibration model built. The Assay results were obtained as below:

Table 2. Assay results for TXA using orthogonal techniques.

Technique	Assay 1	Assay 2	Mean Assay
TRS Raman	101.16	101.70	101.43
HPLC	100.85	101.20	101.03

The orthogonal technique was used for comparison of the Assay results. Using the HPLC technique which is part of the Indian Pharmacopeia monograph, the Assay result was found to be comparable to the results achieved using the Raman technique. This proves the accuracy of the method (Lim et al., 2018).

Greenness measurement

The greenness assessment of the experimental design conducted to determine the content of Tranexamic acid (TXA) in the injection samples was measured using the Agree tool (Pena-Pereira et al., 2020). The report generated is as follows:

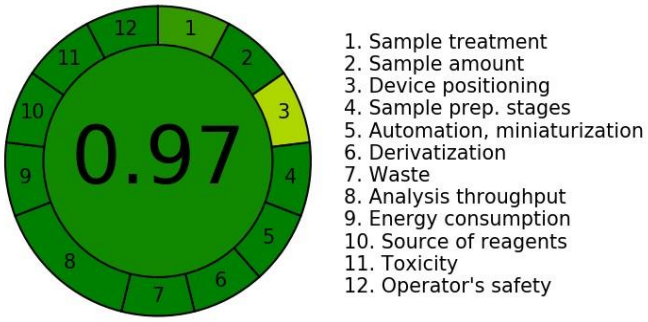

Figure 10. Pictogram representing the overall score for greenness measurement

One way to determine a method's greenness factor is to use analytical greenness metric technique. A thorough, flexible, and easy-to-understand evaluation tool, Analytical Greenness calculator yields a lucid and illuminating outcome (Pena-Pereira et al., 2020). Using a single 0–1 scale, the evaluation criteria are derived from the 12 principles of green analytical chemistry. The ultimate score is determined by applying the principles. The end product is a graphical representation of the

Table 3. Results derived from evaluation criteria of 12 principles of Green Analytical chemistry.

Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.9	1
2. Minimal sample size and minimal number of samples are goals.	1.0	1
3. If possible, measurements should be performed in situ.	0.66	1
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	1
5. Automated and miniaturized methods should be selected.	1.0	1
6. Derivatization should be avoided.	1.0	1
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	1.0	1
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	1.0	2
9. The use of energy should be minimized.	1.0	1
10. Reagents obtained from renewable sources should be preferred.	1.0	1
11. Toxic reagents should be eliminated or replaced.	1.0	1
12. Operator's safety should be increased.	1.0	1

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criteria's weights, the analytical method used to arrive at those weights, and the total score. Table 3 displays the report that was generated using the Analytical Greenness calculator, which was used to calculate the created approach.

In the middle of the pictogram (Figure 10), the overall score of 0.97 is displayed. The dark green values close to one indicate that the technique under consideration is more environmentally friendly. Each criterion's associated color in the section indicates how well the approach performed on that particular evaluation metric. Figure 13 depicts the results for the Green Analytical Chemistry principles, which are fair and acceptable.

Conclusion

TRS is a green and rapid method for assay of tranexamic acid. It is simple to perform, requires minimal sample preparation, and does not use any harmful chemicals. The method is also highly sensitive and accurate. The green concept of this method is based on the non-requirement of any solvent extraction or derivatization steps, which further reduces the environmental impact. The time taken for analysis has been substantially reduced with multiple measurements of the sample within 5 minutes. TRS is an effective tool for determining the Assay of TXA in formulation samples quickly and effectively thus enabling faster and greener analysis.

Overall, TRS Raman spectroscopy using a green concept is a promising new method for the assay of tranexamic acid. It is simple, rapid, sensitive, accurate, and environmentally friendly. A multitude of novel pharmaceutical uses will become possible with the capacity to quickly estimate the concentration of API and produce spectrum-specific data.

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Conflicts of Interest

No conflicts of interest to disclose.

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