Environmental benign analytical quality by design and UPLC method development for Betamethasone and Calcipotriene in ointment

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ABSTRACT
The present study aimed to estimate Betamethasone and Calcipotriene in bulk and ointment formulation using a validated UPLC method by applying analytical quality design with green analytical chemistry principles. The optimal method resulted with Dikma Endeversil C18 ODS (2.1 × 50 mm, 1.8 μm) column at a UV detection of 254 nm encompassed ethanol and potassium dihydrogen phosphate (3.0 pH) buffer 51:49 (v/v), with a flow rate of 0.31 mL/min. The detector response was linear at 12.5–750 and 12.5–75 μg/mL for Betamethasone and Calcipotriene with a detection limit and quantification of 12.48, 37.83 and 3.22, 9.78 μg/mL, respectively. The % recovery was found to be within limits of less than 1.5%. Overall, the AQbD based developed method was greener and confirmed by the greenness evaluation tools. Hence, the optimized technique is environment friendly, simple, robust for the concurrent assessment of Betamethasone and Calcipotriene in bulk and ointment formulation.

1. Introduction
Betamethasone (BMS), also known as Flubenisolone, a synthetic glucocorticoid, changes the gene expression by binding with DNA via glucocorticoid receptor. BMS is used topically to control eczema (inflammatory skin condition) and chemically known as (11β,16β)−9-fluoro-11,17,21-trihydroxy-16-methyl pregna−1,4-diene-3,20-dione (Figure 1) [1,2]. BMS was soluble in water (< 1 mg/mL) and DMSO (79 mg/mL). Calcipotriene (CPT) is chemically (1R,3S,5Z)−5-[(2E)−2-(1R,3aS,7aR)−1-[(1R,2E,4S)−4-cyclopropyl−4-hydroxy-1-methyl but-2-en-1-yl]−7a-methyl octahydro−4H-inden−4-ylidene ethylidene]−4-methyliden cyclohexane−1,3-diol (Figure 1) [1,2]. CPT is D3 analogue used to treat psoriasis. It reduces excessive production of skin cells and reduces psoriasis.

The available marketed ointment formulations containing BMS and CPT are well known for their synergistic effect for treating plaque psoriasis in elderly patients [3]. There is a rising concern across the toxic effect of several chemicals in commercially used analytical methods, especially by the industry, on the environment. Analytical approaches for drug study also include toxic and hazardous solvents with massive volumes of waste...
production and treatment [4]. Nowadays, the pharmaceutical and chemical industries aim to improve an eco-friendly analytical method for drug and chemical analysis with their regulatory commitments by applying the Green Analytical Chemistry (GAC) concepts [5–8]. Eleven out of twelve green analytical principles could be covered [9,10] (sample size, eco-safety chemicals, renewable solvents, waste reduction, \textit{in situ} measurement, miniaturization, hyphenated techniques, multiple analysis, energy consumption, derivatisation, and analyst safety) by utilizing a reliable UPLC instrument.

Analysis of Ointments requires hazardous solvents and may the extract needs to be partitioned between hexane and methanol or methanol/water combinations; the highly lipophilic substance is removed into the hexane layer [11].

To analyses, an ointment, in general, requires a minimum of 50–100 mL of organic solvent. This may raise a serious concern over to the environment and analyst occupational hazards by these chemicals. The exploitation of toxic solvents like methanol, hexane may benefit the analysis but not the environment. So, this raised concern over the development of hazardous methods for the study of pharmaceutical and chemical substances and made to initiate a new approach that is entirely eco-friendly and nullifies the occupational hazard towards the analyst.

There are few analytical methods reported for individual drugs based on varied techniques for detecting BMS and CPT, such as spectrophotometric [12], HPLC [13–17], HPTLC [18], and UPLC [19]. However, few HPLC methods are available for analysing BMS and CPT. Still, they possess disadvantages like using more hazardous chemicals and need to develop AQbD based strategies. That help detects and reduces sources of variability that may result in inadequate method robustness and ensure that the method fulfils its intended performance criteria throughout the product and method lifetime.

As per the literature, no combined UPLC, AQbD, and green analytical-based methodology were available to analyse any drug combinations. Combining the GAC with UPLC and Analytical Quality by Design (AQbD) [20,21] significantly benefits when constructing an innovative method. It creates a synergistic setting for developing environmentally sustainable, effective, and adaptive eco-friendly analytical procedures.

Hence, this work aimed to conduct a novel combined framework for applying GAC principles along with AQbD principles. This novel combination of analytical approach was used for the first instance to develop a green and robust UPLC-PDA study to analyse BMS and CPT in their bulk and marketed ointment formulations. The rotatable central composite design was utilized to determine the critical method parameters and their effect on critical quality attributes of the analytical method.

2. Experimental

2.1. Chemicals reagents

Ortho Phosphoric acid, HPLC grade Ethanol, HPLC grade water (Milli Q or equivalent), Pure BMS, and Pure CPT. (gifted by Glenmark Pharmaceuticals Ltd., Mumbai-India). Marketed formulations Heximar Bointment, with a label claim of 0.05% w/w BMS and 0.005% w/w CPT manufactured by Marini India Pvt Ltd pharma and acquired from the local pharmacy.

2.2. Preparation of stock and calibration solutions

The HPLC grade ethanol made the BMS and CPT standard stock solutions (1 mg/mL). Further, the mobile phase is utilized as a diluent; separate serial dilutions (125-750 and 12.5-75 μg/mL) were achieved using the corresponding standard stocksolutionsfor developing calibration curves. The BMS and CPT mixture was made by adding various aliquot portions from the standard stock solution and filling the volumes with diluent to obtain 500 and 50 μg/mL concentrations.

2.3. Precision and trueness

The six sample solutions for precision and three separate concentration ranges (80, 100, and 120 μg/mL) for
trueness prepared and analysed for intra-day precision and trueness.

2.4. Forced degradation studies

To a series of 10 mL volumetric flasks, containing 1 mL of standard BMS and CPT, then 1 mL of 0.1 N HCl, 0.01 N NaOH, and 3% H₂O₂ were added separately, and made up to volume with diluent. The final solutions were injected into the UPLC system for the chromatographic analysis, with a time range of 0, 30 min, and 1 h and the amount of degradation was compared with the control.

3. Photodegradation

Standard solutions of BMS and CPT were prepared and placed in Ultraviolet rays for 3 h and injected into the UPLC system for studying the photodegradation studies.

3.1. Assay of pharmaceutical formulation

An accurately weighed sample from the tube’s top, middle, and bottom positions, equivalent to 5 and 0.50 mg of BMS and CPT, respectively, has been transferred to a 10 mL volumetric flask. The HPLC grade ethanol (5 mL) was added, sonicated for 15 min, makeup with diluent, and filtered through 0.45μ Polyvinylidene fluoride (PVDF) filter. Final Aliquots of 4 μL infused in as triplicate, the described earlier procedures have been used to determine the concentration of the investigated drugs in their formulations.

4. Results and discussion

Although pharmaceutical researchers have established several analytical techniques for evaluating drugs and chemical substances existing in various samples, most of these methodologies possess environmental and health consequences when transferring a method to commercial exploitation on an industrial scale. Devel- oping a strategy should focus on enriching eco-friendly concepts so that it helps the method to sustain for long-term usage. The preliminary step in the liquid chromatographic methods was the selection of the most appropriate solvents. There are several agencies [22,23] that have listed the solvent-based on the cumulative energy demand (CED) calculations. Still, all these solvents cannot be applied in liquid chromatography due to several constraints like solvent compatibility with either chemicals (solubility) or instruments (high noise). From these constraints, very few solvents were picked for the column chromatography, like ethanol, a good alternative for methanol, propylene carbonate for acetonitrile, and ethyl lactate for ethyl acetate. From the initial trials of the solubility of the drugs, it is found that the drugs were completely soluble in ethanol. So, ethanol, a biodegradable solvent with significantly less CED, has been selected for further analysis.

Selecting a pH for separating two analytes is the primary task for an analyst, and it is based on two main criteria. Firstly, column-like silica-based type should be operated at a pH range of 2-8. No decrease or increase in the pH outside this range will damage or solubilize the bonded silica phase. The second criteria are based on the analyte’s need to be separated, where BMS and CPT have a pKa value of 13.4 and 14.39 and ionize in lower pH (acidic range). The basic principle of chromatography states that the ionized form of analytes is more polar and less retained in the reverse phased columns, so the mobile phase pH selected at 3.0 to elute analytes with short runtime to enhance the green analytical principles.

The next factor for consideration was the composition of the two drugs as pharmaceutical dosage form of BMS and CPT was in a ratio of 1: 10. There should be a definite check in the interaction of conditions and parameters with the responses. So instead of checking the parameters and their interaction with each other by using one factor at a time and wasting the solvent, energy, and time AQD technique will simplify this problem and helps to find the interaction between the constraints and responses.

Considering this, new approaches have been developed to integrate UPLC, AQbD, and GAC concepts into a coherent model to improve the long-term sustainability and robustness of BMS and CPT estimation. Following the five components of the AQbD paradigm makes it possible to understand method parameters and their relationships better, identify elements that significantly influence performance characteristics, and allocate permissible limits of variation. In this article, a thorough overview of AQbD, with GAC principles, has been applied to develop a sustainable analytical procedure to estimate a drug combination BMS and CPT, demonstrating the technological advances in a chemical compound or drug analysis.

4.1. Identifying attribute and risk assessment

The Analytical Target Profile (ATP) contains most of the attributes necessary to validate the analytical method’s quality and purpose. Table S1 illustrates the numerous chromatographic components that comprise the ATPs required for effective method development. The Critical Quality Attributes (CQAs) are often detected during the formulation development process as a part of Quality by Design-based manufacturing process characteristics. In AQbD assessment, resolution, precision, retention, peak shape, and drug sensitivity are probable CQAs. The Ishikawa Fishbone Diagram is utilized to identify risks (Figure 2); risk evaluation is the fundamental ideology with varying criteria on various responses. This approach helps investigate the cause-and-effect
relationship of the experimental conditions in our study design; thus, much less work and time were consumed, and quicker optimization processes were attainable.

The separation criticality among a set of closely eluted peaks (BMS and CPT) was investigated using isocratic mobile phase elution by trails of the one-time factor were utilized and found that second compound peak tailing (PT2), emphasizing the tailing of these combinations. Trails also show that variation of other factors like temperature, injection volume leads to a negligible effect on CMP’s. Few materials attribute like flow rate, pH, and mobile phase composition are considered significantly impacting the parameters.

Consequently, minor changes in the above factors significantly affect the output in the system suitability parameters. The essential CAAs were chosen, taking into account factors such as resolution (Rs), second peak tailing (PT2), and the retention time of the second peak (RT2) were considered as critical attributes for the present method based on the one-time factor analysis. A rotatable central composite design was applied for the method development ensures overall consistency of prediction error and attained through the proper choice of \( \alpha \) values gives more detailed interaction ranges and the effect of CMP on CAA.

4.2. Experimental design

4.2.1. Rotatable central composite design (rCCD) applied methods optimization

The entire optimal state was evaluated with rCCD, the combination when defining its design space for rigorous analysis; the three CMPs were constrained to pilot study examination using rotatable central composite design (rCCCD). The preliminary analysis determined the levels of each parameter, and the chosen CAAs were employed to get the best results. (Table S2). At the same time, high ethanol percentages (55% for the mixture) and flow rates (0.35 mL/min) improved peak shape but negatively impacted peak resolution. Furthermore, at low ethanol (45%) and low pH 2.5, a resolution has been achieved with a longer run time which is not recommended according to green analytical principles. The mixture was measured in three separate ranges for ethanol % \((-\alpha, 0, +\alpha)\), pH \((-\alpha, 0, +\alpha)\), and flow rates \((-\alpha, 0, +\alpha)\). Six centre points for accurate experimental error estimation and the resulting RT2, Rs, and PT2 values were recorded after a sequence of twenty experiments were performed in chronological order. After the generated frameworks were analysed, the method was discovered to obey polynomial equations with well-considered linear effects, quadratic effects, and factor interactions (Table S3).

4.2.2. Mathematical validation, interpretation, and desirability function

The predictive viability of the regression models for the three responses was then ensured with the obtained ANOVA data, lack of fit non-significance, reasonable standards of \( R^2 \), adjusted and predicted \( R^2 \) (Table S3). Graphical data interpretation techniques such as Perturbation, contour, and 3D surface plots were used to investigate the impacts of CMPs like ethanol (X1), Flow rate (X2) and pH (X3) on retention of the second peak (Y1), Resolution (Y2) and Tailing factor of the second peak (Y3) (CAA’s). The model’s \( p \)-value of \(< 0.0001\) for (Y1,2,3). The Model F-values of 1444.94 (Y1), 72 (Y2), 29.76 (Y3) implies the model is significant.
only a 0.01% chance that an F-value this large could occur due to noise. *P*-values less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB, AC, A\(\cap\)2, B\(\cap\)2, C\(\cap\)2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-values of 3.59, 2.26, and 3.22 imply a 10.36%, 19.58%, and 11.26% chance of a Lack of Fit. The obtained non-significant lack of fit is good as the model can fit for the further steps. The Predicted R\(\cap\)2 of all three interactions reasonably agrees with the Adjusted R\(\cap\)2, i.e. a difference of less than 0.2 indicates a good precision measurement of signal-to-noise ratio. The ratio of 131.351, 28.704, and 19.386 indicates an adequate signal and shows that the model can navigate the design space. The Perturbation, contour plots, and 3D (Figure 3) and second-order polynomial equations Table S4 demonstrate that changing the CMA in the design space shows a prominent effect on three attributes and the need to combine these variations in the determination of BMS and CPT. CMA’s role on CMP’s as increasing in ethanol concentration increases the RT2, indicating that CPT retains in the column on the increase in ethanol concentration and showed a steep rise in Rs but decreased in PT2. The second-factor flow rate shows increased RT2 and Rs but showed a parabolical decrease in PT2. Finally, pH as the third factor impacts RT2 as an increase in pH increases RT2 and resolution but had a minimum effect on the PT2.

The present study’s objective was to establish a procedure that did not affect green analytical principles and was primarily focused on waste reduction via UPLC to avoid interfering with validation standards, as previously stated. The obtained results with a PT2 limit of less than 2 for an acceptable peak elution, the ethanol ratio, flow rate, and pH were maintained, which might not affect PT2. Regarding RT2, even if ICH guidelines impose no limits, but according to GAC principles, the
delayed RT$_2$ was considered. Finally, there is a criterion for the resolution, which must be more than 1.5. The three responses were interconnected, demonstrating the need to select a technique that did not interfere with the other responses. Derringer’s desirability (Table S5) methodology was used to determine the ideal combination of circumstances based on the importance and limitations of each response. For instance, as seen in Figure 4, the desirability methodology proved specific goals within predefined criteria. Some of the parameters like pH were set as a target. The resolution responses were kept maximum for better separation, and the tailing factor was developed to minimize to attain better elution. A specific experimental range was evaluated for compositions containing an ethanol ratio of 52.04 with a flow rate of 0.318 and buffer pH of 3 showed desirability of 0.832, which is near to 1. The same parameters were selected as a target result which was compared practically and showed a deviation of less than 10% that met the set limitations to the entire degree possible, which would have been the limit of consistency. The overlay plot for the design space was depicted in Figure 5, which shows any changes in the yellow region do not require further revalidation. The following parameters were chosen for the final optimum RP-UPLC chromatographic setup: mobile phase is ethanol and buffers 51:49, flow rate (0.31 mL/min), and pH 3.0.

4.3. Validation of the proposed method

4.3.1. Specificity

The technique’s specificity was tested by injecting a laboratory-prepared extracted placebo to verify the lack
of interference with the analyte’s elution. The absence of additional peaks in the chromatogram demonstrates that the approach was specific for drug analysis. Figure 6 shows the blank, placebo, and Peak purity plot chromatograms of the BMS and CPT method.

4.3.2. System suitability parameters
After six duplicate injections, the system suitability tests indicated no statistically significant difference between BMS and CPT in peak area, retention time, theoretical plates, and peak tailing. The % RSD values were determined to be < 1.5%, validating the high degree of trueness of the chromatographic instrument. The validation results for system specificity are summarized in Table 1.

4.3.3. Linearity LOD and LOQ
The linearity for BMS and CPT was determined by constructing calibration curves in the concentration ranges of 125–750 and 12.5-75 μg/mL, respectively (Figure 7). Each solution of different concentrations was injected in triplicates, and peak areas were observed for each solution. The calibration curves were constructed by plotting the mean area of a peak versus the concentration (μg/mL). The correlation coefficient results suggested that the linearity was excellent in this case (0.999 for BMS and 0.9992 for CPT). The regression models were derived in the way shown in Table 1. The LOD and LOQ of the approach were determined theoretically to be 12.484, 37.831 μg/mL for BMS, and 3.229, 9.785 μg/mL for CPT, respectively, and then applied in practice.

4.3.4. Precision and trueness
The precision has been calculated at three different concentrations, and the results are portrayed in Table 1. The % RSD for inter-day and Intra-day precision ranges from 0.59, 0.70 and 0.48, 0.49 for BMS and CPT. The trueness of the sample was validated at three different concentrations of 80, 100, and 120%; results were
Figure 6. Chromatogram for (a) placebo without drugs (b) blank chromatogram and (c) peak purity plots for BMS and CPT.

Figure 7. Linearity graphs for (a) BMS and (b) CPT and standard chromatogram for BMS and CPT at a concentration of 500 and 50 μg/mL.
Table 1. Validation results for BMS and CPT.

| PARAMETERS                        | BMS          | CPT          |
|-----------------------------------|--------------|--------------|
| Linearity Range ([μg/mL])         | 125–750      | 125–75       |
| Slope [μg/mL]                     | 7689.06 ± 1.65 | 373.491 ± 0.665 |
| Intercept (μg/mL)                 | 73385.94 ± 165.3 | 2380.95 ± 38.99 |
| Correlation coefficient           | 0.9999       | 0.9990       |
| System suitability parameters CI  | 37045.08     | 1818.06      |
| for intra-day                     | 12.48        | 3.229        |
| n(observations)                  | 37.831       | 9.785        |
| Trueness Mean ± RSD               | 99.87 ± 0.13 | 99.58 ± 0.70 |
| Alpha %RSD                        | 0.05         | 0.05         |
| CI                               | 0.0838       | 0.4568       |
| Precision CI                     | 98.789 ± 0.62 | 99.359 ± 0.861 |
| % RSD                            | 0.631        | 0.866        |
| n observations                   | 18           | 18           |
| CI for intra-day                 | 0.5535       | 0.46         |
| CI for inter-day                 | 0.288        | 0.48         |
| System suitability parameters CI  | 4455.8 ± 66.14 | 2957 ± 23.6  |
| No. of theoretical plates        | 1.48         | 0.81         |
| % RSD                            | 0.7556 ± 0.01 | 1.154 ± 0.01 |
| % RSD                            | 0.67         | 0.66         |
| Peak area Mean ± RSD             | 851670 ± 190.47 | 38915.2 ± 371.34 |
| % RSD                            | 0.02         | 0.95         |
| Resolution Mean ± RSD            | 1.18 ± 0.01  | 1.57 ± 0.02  |
| % RSD                            | 1.10         | 0.81         |

Mean of six determinations, ± Set of five determinations, *** Set of three determinations

5. Forced degradation results

The forced degradation studies of BMS and CPT were studied in various stressed conditions and found to be stable in acid condition with around 5%. BMS degrades in the alkali with more than 15% and CPT degrades up to 15% in oxidation however, the other drugs (CPT in alkali and BMS in Oxidation) shows to be stable in those conditions. Photo degradation results illustrate that the drugs were stable and shows less than 2% degradation after 3 h of exposure to the UV light. Figure 8a, b, c, d and Table 2 illustrates the total degradation of the two drugs in their respective stressed conditions.

5.1. Assay of marketed formulation

The efficacy of the newly developed UPLC method was proven by examining the mixture of commercially available pharmaceutical products (Figure 7). The drugs, as mentioned above, were estimated in such a selective manner, with high recovery values in their combination formulation. Furthermore, the efficacy of the extraction and the relationship between the excipients were investigated utilizing the conventional addition methodology. The findings reveal that satisfactory recoveries were achieved with no influence from the common ointment excipients used in the preparation. The student’s t and F-tests were used to compare the findings obtained by the designed approach to the results obtained by other methods for assessing each of the studied substances. None of the obtained results was more significant than any calculated results, indicating no statistically significant variations in the two processes’ trueness and precision (Table 3).

5.2. Greenness assessment and methods comparison

The GAC offered many advancements for creating stable green analytical methodologies that would be implemented in the future. As part of the 3R principles, hazardous/toxic chemicals are substituted with more environmentally friendly and cost-effective alternatives, or their usage was regulated if excessive (Replace, Reduce, Reuse) [24]. Compared to prior methodologies for analysing drug mixtures, this developed method effectively measured the BMS and CPT in their combination by employing greener ethanol rather than the potentially hazardous methanol or acetonitrile, which was widely used. This suggested UPLC technique uses the least amount of solvent and energy possible. As a result of these characteristics, the proposed methodology seemed more ecologically sustainable than one that had already been reported. Five state-of-the-art metrics were employed to determine if a device was ecologically sustainable: NEMI, GAPI, Analytical Eco-Scale, AMGS, and AGREE.

NEMI is a statistic that measures how environmentally friendly a method is. NEMI pictogram is a classic tool for assessing greenness in the technique. NEMI results were represented as a circular pictogram parted into four quadrants with a colour coding with green and colourless and the detailed assessment was explained in supplementary file. Table S6 shows the NEMI pictogram for the proposed method.

GAPI results represent the colour coding as green, yellow, and red. GAPI covers sample preparation and instrumentation assessment which is a drawback in NEMI [25] detailed explanation was given in supplementary file. Table S6 portrayed the GAPI pictograms for the proposed method and reported using the help of J. Plotka-Wasyłka [26] developed software for easy and perfect projections of the GAPI assessment [27].

Analytical eco-scale [28] is signified through a final score of 100. The final score was reduced by the Penalty point (PP) based on the method performance like the chemicals, reagents, instruments, and wastage by the method is considered and deducted. This process involves five different steps; as it was designed for our developed UPLC methodology was well explained in supplementary file.

The approach with a 75 was deemed greener; the recommended approach received a score of 96,
reflecting the approach’s influence on future use in terms of environmental acceptability.

AMGS amalgams some metrics, including SHE (safety, health, and environmental assessment). It is used to estimate solvent safety using the geometric mean, CED (Cumulative Energy Demand), and AMVI (Analytical Mass Volume Intensity) [29], used for calculating solvent volume waste and instrument and solvent selection. It is a semi-quantitative method for determining environmental sustainability [30]. Once the data required has been administered, it generated a score of 49.38 (Table S6), indicating that the technique’s ecological impact is eco-friendly as the decrease in score indicates the greener method.

AGREE metrics is a software-based program based on the 12 principles. Results incorporated in each principle or slot show the impact of the method on the environment, with a score of 1 indicates the process
greenest [31–33]. The total score of 0.89 in Table S6 shows that the proposed method was the greenest in all respects. In retention time or the waste produced, the greenest results were correlated with the reported HPLC techniques. Table S6 provides comparative findings of the procedures that have been published and those suggested for BMS and CPT.

6. Conclusion
The incorporation of the AQbD approach with GAC postulates a new eco-friendly and precise analytical methodology. For analysis of two drugs, BMS and CPT, in their bulk and branded formulations, established a novel green AQbD system. A detailed step-by-step overview of this AQbD approach was introduced to provide an accurate image of system variables and develop simple, stable, and robust methods that can be utilized in QC laboratories without further revalidation. Experiment designs like the rotatable central composite design were used for mathematical optimization trials with mobile phase composition, flow rate, and buffer pH as variables and resolution, retention time, and peak tailing as a response. Instead of toxic organic solvents including methanol and acetonitrile, less hazardous ethanol-based mobile phases were used. In UPLC, shorter columns with the finest particle sizes at high pressure allowed for a 2.4-minute analysis indicating that the developed method was much better than previously reported methods in retaining two drugs within less than 1.14 min without compromising the ratio factor. The findings identified the optimum working conditions for the drugs mix and design spaces, experimentally tested with extra runs. The optimized method was further validated and found the trueness results at three different levels, and precision results show that less than 1.5% RSD was within limits. Linearity range and correlation coefficient, which is less than one and under limits. Finally, the green assessment tools results demonstrated that the approach was the most environmentally sustainable and could be readily adapted for industrial and quality control purposes.

Disclosure statement
No potential conflict of interest was reported by the authors.

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References
[1] Wisher D. Martindale: the complete drug reference. 37th ed. Medical Library Association; 2012. doi:10.3163/1536-5050.100.1.018.
[2] O’Neil MJ, Heckelman PE, Dobbelaar PH, et al. The merck index: An encyclopedia of chemicals, drugs, and biologicals. Hoboken (NJ): The Royal Society of Chemistry; 2013.
[3] Vakirlis E, Kastanis A, Ioannides D. Calcipotriol/Betamethasone Dipropionate in the treatment of Psoriasis Vulgaris. Ther Clin Risk Manag. 2008;4:141–148.
[4] Celia C, Di Marzio L, Locatelli M, et al. Current trends in simultaneous determination of Co-administered drugs. Separations. 2020;7(2), doi:10.3390/separations7020029.
[5] Tobiszewski M, Namieśnik J. Greener organic solvents in analytical chemistry. Curr Opin Green Sustainable Chem. 2017;5:1–4, doi:10.1016/j.cogsc.2017.03.002.
[6] Winterton N. Twelve more green chemistry principles. Green Chem 2001;3(6):G73–G81, doi:10.1039/B110187K.
[7] Kirchhoff MM. Promoting sustainability through Green chemistry. Resour Conserv Recycl. 2005;44(3):237–243, doi:10.1016/j.resconrec.2005.01.003.
[8] Kanaka Parvathi K, Sugumaran A, Hemanth Kumar C, et al. Environmental impact of greenness assessment tools in liquid chromatography – A review. Microchem. J. 2021; doi:10.1016/j.microc.2021.106685.
[9] Badami BV. Concept of Green chemistry. Resonance. 2008;13(1):1041–1048, doi:10.1007/s12045-008-0124-8.
[10] Anastas PT, Warner JC. Green Chemistry : Theory and Practice. Oxford [England]; New York: Oxford University Press; 1998.
[11] Yacobi A, Shah VP, Bashaw ED, et al. Current challenges in bioequivalence, quality, and novel assessment technologies for topical products. Pharm Res 2014;31(4):837–846, doi:10.1007/s11095-013-1259-1.
[12] Mahmood A, Rapalli VK, Waghule T, et al. UV spectrophotometric method for simultaneous estimation of Betamethasone valerate and tazarotene with absorption factor method: application for in-vitro and Ex-vivo characterization of lipidic nanocarriers for topical delivery. Spectrochim Acta Part A Mol Biomol Spectrosc. 2020;235:118310, doi:10.1016/j.saa.2020.118310.
[13] Singh M, Charde M, Shukla R, et al. Determination of Calcipotriene in Calcipotriene cream 0.05% w/w by RP-HPLC method development and validation. Res J Pharm Technol. 2011;4:1219–1223.
[14] Swapna B, Kiran G, Vasudha B, et al. Investigation of an adsorbent based on novel starch/chitosan nanocomposite in extraction of indigo carmine dye from aqueous solutions. Biointerface Res Appl Chem. 2020;10(3):5556–5563.
[15] Bhogadi RK, Satyanarayana A, Rao NS, et al. Stability indicating RP-HPLC method for estimation of impurities of vitamin D3 analogue and corticosteroid used as antipsoriatic drugs. An attempt to characterize Pre-calcipotriene. Am J Anal Chem. 2015;06(13):1050–1058, doi:10.4236/ajac.2015.613100.
[16] Roy C, Chakrabarty J, Ratti RR. Development and validation of a stability-indicating NP-HPLC method for simultaneous determination of Betamethasone dipropionate and Calcipotriene in topical dosage form. Arch. Appl. Sci. Res. 2013;5(2):15–24.
[17] Ponnnusamy C, Krishnaswami V, Sugumaran A, et al. Simultaneous Estimation of Artemisinin and Dexamethasone in Nanodispersions and Assessment of Ex-Vivo Corneal Transport Study by RP-HPLC. Curr Pharm Anal 2014;10(1):44–50, doi:10.2174/157341291001014010211031.
[18] Merley HA, El-Mosallamy SS, Hassan NY, et al. Validated chromatographic methods for simultaneous determination of calcipotriol monohydrate and Betamethasone.
dipropionate in the presence of Two dosage form additives. J Chromatogr Sci. 2019;57(4):305–311. doi:10.1093/chromsci/bmy112.

[19] Chen M-Y, Tang Y-J, Wang Y-C, et al. Quantitative determination of Betamethasone sodium phosphate and Betamethasone dipropionate in human plasma by UPLC-MS/MS and a bioequivalence study. Anal Methods. 2016;8(17):3550–3563. doi:10.1039/C6AY00202A.

[20] Srinubabu G, Raju A, Sarath N, et al. Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design. Talanta. 2007;71(3):1424.

[21] Chanduluru HK, Sugumaran A. Eco-Friendly estimation of isosorbide dinitrate and hydralazine hydrochloride using Green analytical quality by design-based UPLC method. Talanta. 2021;11(45):27820–27831. doi:10.1039/D1RA04843K.

[22] Prat D, Pardigon O, Flemming H-W, et al. Sanofi’s solvent selection guide: A step toward more sustainable processes. Org Process Res Dev. 2013;17(12):1517–1525. doi:10.1021/op400256S.

[23] de la Guardia M, Garrigues S. Green chemistry series. Present and Future Green Anal Chem. 2020: 1–18. doi:10.1039/978178016148-00001.

[24] EL-Shorbagy HI, Elsebaei F, Hammad SF, et al. Optimization and modeling of a Green dual detected RP-HPLC method by UV and fluorescence detectors using two level full factorial design for simultaneous determination of sofosbuvir and ledipasvir: Application to average content and uniformity of dosage unit testing. Microchem J. 2019;147:374–392. doi:10.1016/j.micchem.2019.03.039.

[25] Plotka-Wasyli J. A New tool for the evaluation of the analytical procedure: Green analytical procedure index. Talanta. 2018;181:204–209. doi:10.1016/j.talanta.2018.01.013.

[26] NFPA. List of nfpa codes and standards https://www.nfpa.org/Codes-and-Standards/All-Codes-and-Standards/List-of-Codes-and-Standards (accessed Apr 6, 2021).

[27] Van Aken K, Strekowski L, Patiny L. EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters. Beilstein J Org Chem. 2006;2:1–7. doi:10.1186/1860-5397-2-3.

[28] Hartman R, Helmy R, Al-Sayah M, et al. Analytical method volume intensity (AMVI): A Green chemistry metric for HPLC methodology in the pharmaceutical industry. Green Chem. 2011;13(4):934–939. doi:10.1039/c0gc0524j.

[29] AMGS. AMGS spreadsheet https://www.acsgcipr.org/amgs/ (accessed Apr 9, 2021).

[30] Pena-Pereira F, Wojnowski W, Tobiszewski M. Agree-analytical GREEnness metric approach and software. Anal. Chem. 2020;92(14):10076–10082. doi:10.1021/acs.analchem.0c01887.

[31] Chanduluru HK, Sugumaran A. Estimation of pitavastatin and ezetimibe using UPLC by a combined approach of analytical quality by design with green analytical technique. Acta Chromatogr. 2021. doi:10.1556/1326.2021.09949.

[32] Kannaiah K, Sugumaran A. Eco-Friendly multivariant green analytical technique for the estimation of ketoconazole by uv spectroscopy in bulk and cream formulation. Quimica Nova. 2021; doi:10.21577/0100-4042.2017.0798.