RESEARCH ARTICLE

Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Adapalene and Quercetin

Chaudhari Pallavi Manojkumar*, Bhotkar Priyanka Rangrao

Department of Pharmaceutics, Dr. D.Y. Patil Pratishthan's, Dr. D.Y. Patil College of Pharmacy, Akurdi, Affiliated to Savitribai Phule Pune University (SPPU), Pune, Maharashtra-411044, India

Received: 28th Aug, 2025; Revised: 7th Sep, 2025; Accepted: 12th Sep, 2025; Available Online: 25th Sep, 2025

ABSTRACT

Background: The UV Spectrophotometric method is quick, cost-effective, accurate simple, rapid and precise was developed and validated for simultaneous estimation of Adapalene and Quercetin. Adapalene having anti-acne activity, Comedolytic effect, Keratolytic effect, and Quercetin having anti-inflammatory, Antibacterial, Wound healing activity

Purpose: To develop and validate simultaneous equation method for Adapalene and Quercetin.

Materials and method: This method involved estimation using two wavelengths. Since the absorbance of Adapalene and Quercetin reaches its maximum at 320 and 370 nm, respectively, these wavelengths were used to measure the absorbance and to estimate their relative concentrations. Adapalene and Quercetin both follow Beer-Lambert's rule at concentrations between 2-10 µg/mL.Dimethyl sulfoxide (DMSO) was used as common solvent.

Result: This method was validated with respect to Linearity, accuracy, precision, Limit of Detection and quantification as per ICH norms. %RSD was found to be less than 2. Linear regression coefficient for Adapalene and Quercetin was found to be 0.9987 and 0.9983 respectively. For Adapalene, the LOD and LOQ values attained are 0.111 µg/mL and 0.33µg/mL. Also, the LOD and LOQ values attained for Quercetin are 3.40 µg/mL and 3.16 µg/mL, correspondingly. Thus, this method was found accurate, precise, and reproducible for simultaneous estimation of Adapalene and Quercetin

Keywords: Adapalene, Quercetin, UV-Spectrometry, Simultaneous estimation, Vierordt's techniqu

How to cite this article: Chaudhari Pallavi Manojkumar, Bhotkar Priyanka Rangrao. Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Adapalene and Quercetin. International Journal of Drug Delivery Technology. 2025;15(3):1090-93. doi: 10.25258/ijddt.15.3.25

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Adapalene: is a synthetic derivative of naphthoic acid, a member of the retinoid class. ADP's structural components, methoxyphenyl and adamantane (tricyclo [3.3.1.1] decane), these chemical groups assign certain biological and physicochemical characteristics. (Figure 1(a)) 6-[3-(1-adamantyl)-4-methoxyphenyl] naphthalene-2-carboxylic acid is the chemical name. having many pharmacological activity Anti-Inflammatory, Comedolytic Effects Keratolytic effect, Immunomodulatory effect, Depigmenting effect and Antibacterial Activity[1].

Quercetin: is flavonoid from plant that found in vegetables, fruits, seeds and leaves structurally Quercetin is (Figure1 (b)-(3, 4-dihydroxy phenyl)-3, 5,7-trihydroxychromen-4-one[2]. Quercetin has many health benefits that are promising for the skin, including antibacterial, anti-inflammatory, antifungal, antiviral, anti-aging, anti-osteoporosis, anti-cancer, anti-itching, anti-psoriasis, skin whitening, wound healing, and photoprotection. It also has a high level of antioxidant activity[3].

The primary aspect of the pharmaceutical development program is the development of analytical methods. Additionally, it involves demonstrating that the developed method can be utilized to determine the concentration or amount of API in different formulations[4]. One of the earliest instrumental methods of analysis is UV-visible spectrometry, which serves as the foundation for a number of excellent approaches for figuring out the micro and semi-micro amounts of analytes in a sample. The study's specific goal was to create and verify a method using UV spectrophotometric for the simultaneous measurement of quercetin and adapalene[5].

Ultraviolet (UV) visible spectroscopy is one of the often-active technique in pharmaceutical analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Adapalene was gift sample from Glenmark pharmaceuticals Pvt. Ltd Nashik, Quercetin was purchased from New Neeta chemical's lab. Dimethyl sulfoxide purched from Fine chemicals Mumbai

Instrumentation

UV Spectrophotometer Shimadzu UV-1900i, equipped with Quartz Cuvette of 1 cm was used. A Weighing balance LC GC AS 220/C/2 was used for weighing of drug and chemicals.

Selection of Solvent

According to Indian Pharmacopoeia 2018 guidelines for choosing a common solvent, we tested the solubility of drug in variety of solvents. Ethanol, methanol, and dimethyl sulfoxide solvents were used to test solubility. For the analysis of both Adapalene and Quercetin using the

**Author for Correspondence: pallavichaudhari@dyppharmaakurdi.ac.in*

suggested approach, dimethyl sulfoxide (DMSO) was determined to be the common solvent.

Determination of λ Max

Preparation of Stock Solution

Adapalene and Quercetin solution of stock was formulated in adding 10 mg of drug in 100 ml of DMSO that gave 100µgm/ml respectively.

Preparation of Working Solution

Using the stock solution that was previously prepared a10 ml volumetric flask was filled with 1 ml of each drug solution, and the volume was attuned with DMSO to reach 10 µg/ml. The UV-Vis Spectrophotometer was then used to scan the sample between 200 and 400 nm against DMSO. The wavelength that corresponded to the highest absorbance, or its λ-max, was then recorded, which was at 320 nm for Adapalene also 370 nm for Quercetin, respectively.

Calibration Curve Preparation

Standard working solution (2,4,6,8) and 10 µg/ml for each medication were made and scanned across the 400–200 nm UV spectrum. Using the x-axis for concentration and the y-axis for absorbance, a calibration curve for concentration vs. absorbance was created, displaying a straight line. In the 2–10 µg/ml concentration range for both medications, this straight-line exhibited linearity. Adapalene and quercetin were shown to have correlation coefficients of 0.9989 and 0.9983, respectively.

Simultaneous Equation Method

Drug estimate at the same time is a crucial technique since the new mixed formulation has been approved for sale. Since a high dose can result in overdose side effects and a low dose will prevent the patient from receiving the recommended dosage, the primary goal of quantitative assessment is to confirm whether a given medication contains the same amount of substance as stated[6]. Whenever two pharmaceuticals or additional than two drugs are included in a combination dosage form in the medication combination, Vierordt's technique, also known as the simultaneous equation, was applied to that formulation[7,8].

Finding the concentration of various components in a given mixture by solving a set of problems is one of the most popular and straightforward techniques used for spectrophotometric multicomponent analysis. simultaneous equations, notwithstanding the overlap in their spectra[8]. The additive nature of each component's absorbance in any mixture serves as the foundation for this technique. As referred by overlain spectra (Figure 2) Two wavelengths,

Table 1: Result of validation parameter

Parameter	Adapalene	Quercetin
λmax (nm)	320	370
Range Linearity (µg/ml)	2-10µg/ml	2-10µg/ml
Equation Linearity	y = 0.0645x + 0.0089	y = 0.0323x - 0.0006
Correlation coefficient	0.9989	0.9983
Slope (b)	0.0645	0.0323
Intercept (a)	0.0089	0.0006
LOD	0.112	3.40
LOQ	0.33	3.16

Data represented as mean ± SD (n=3)

Table 2: Accuracy study

Drug concentration (µg/ml)	% Recovery	% Recovery
Adapalene	80	99.20 ± 0.0040
	100	99.48 ± 0.0024
	120	101 ± 0.0066
Quercetin	80	99.58 ± 0.0021
	100	99.11 ± 0.0032
	120	112 ± 0.0042

Data represented as mean ± SD (n=3)

320 nm for Adapalene and 370 nm for Quercetin, were chosen as the working wavelengths of Adapalene (10µg/ml) and Quercetin (10µg/ml), at which both medications demonstrated absorbance for one another.

These two drugs absorptivity was measured at 320 nm and 370 nm. Using the absorptivity values from equations (1) and (2) at specific wavelengths, a set of double simultaneous equations was created.

$Cx = (A2ay1\text{-}A1ay2)/ (ax2ay1\text{-}ax1ay2)$ ------ (1)[9]

$Cy = (A1ax2\text{-}A2ax1)/ (ax2ay1\text{-}ax1ay2)$ ------ (2)[9]

Were,

Adapalene and quercetin concentrations (µg/ml) in a known sample solution are denoted by Cx and Cy, respectively.

The absorbance of sample solutions at 320 and 370 nm is denoted by A1 and A2, respectively. ax1 and ax2 are absorptivity of Adapalene on 320 nm and 370nm, ay1 and ay2 are denoted absorptivity of Adapalene 320nm and Quercetin at 320 nm. The correctness of the equation was confirmed by using a mixed standard of pure drug samples of binary pharmaceuticals, determining their absorbance at the proper wavelength, and computing the concentration of binary components.

Developed Method Validation

Linearity

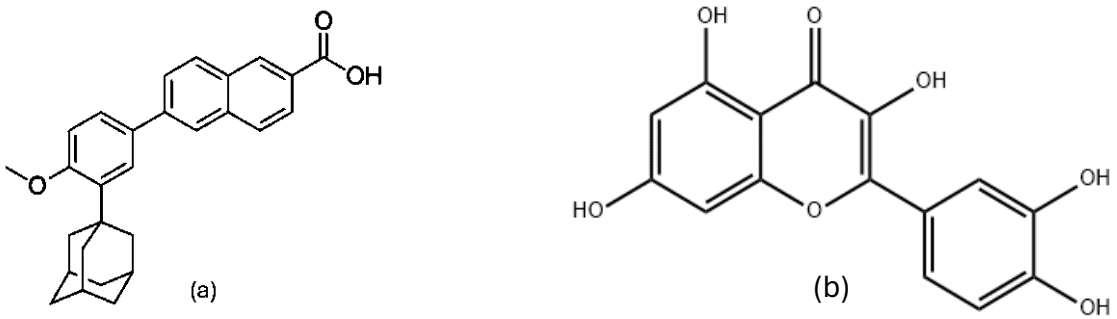

Figure 1: Structure of (a) Adapalene; (b) Quercetin

According to the established procedures, suitable dilutions of standard stock solutions were tested for every medication. For both medications, the concentration range was determined to be 2–10 µg/ml.by Beer-Lambert (Table 1) displays the method's linearity data.

Accuracy

When test results match the actual value, it is said to be accurate[10]. Three different level of recovery experiments were carried out using the conventional addition technique. (80%, 100%, and 120%) in order to examine the correctness of the suggested approach. A standard drug solution was added to a sample solution that had already been examined, and the percentage of drug content was then determined, subsequent to the conventional addition, the total drug concentration, or Ct, is measured. Ca denote the concentration of drug added to the formulation, and Cs represents the concentration of drug in the formulation sample., the percentage The additional pure drug's recovery was computed as % retrieval= [(Ct-Cs)/Ca] x 100[11] (presents) the findings from the recovery investigations.

Precision

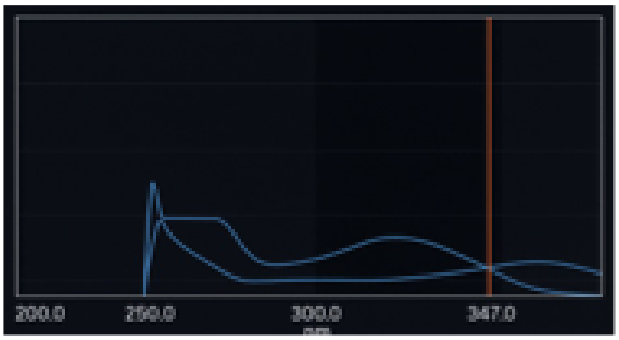

Figure 2: Overlay of maximum absorption of Adapalene and Quercetin

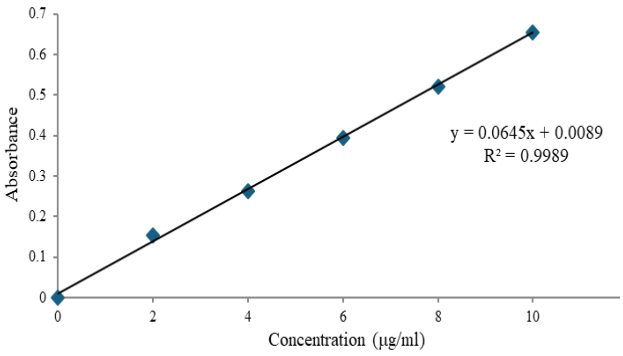

Figure 3: Adapalene Calibration curve

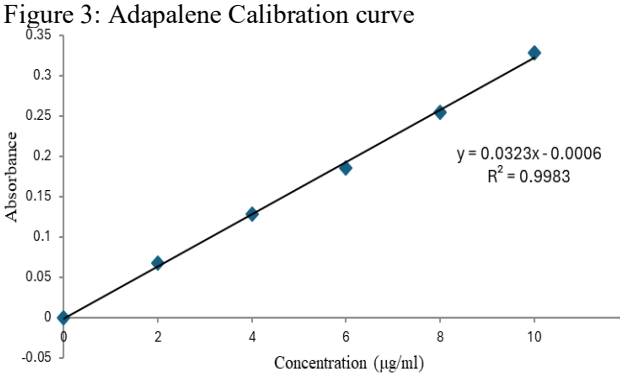

Figure 4: Quercetin Calibration Curve

Table 3: Inter-day precision

Precision	Mean absorbance	Standard deviation	%RSD
Adapalene	0.435	0.015	0.234
Quercetin	0.163	0.047	0.935

Data represented as mean ± SD (n=3)

Table 4: Intra-day precision

Precision	Mean absorbance	Standard deviation	%RSD
Adapalene	0.432	0.015	0.234
Quercetin	0.157	0.047	0.968

Data represented as mean ± SD (n=3)

Table 5: study of Ruggedness

Ruggedness	Adapalene		Quercetin	
	Mean ± SD	%RSD	Mean ± SD	%RSD
Analyst 1	0.435 ± 0.0013	0.23	0.162 ± 0.004	0.8
Analyst 2	0.436 ± 0.006	0.25	0.163 ± 0.003	0.7

Data represented as mean ± SD (n=3)

Inter-day and Intra-day precision the formulation analysis, which was conducted six times with the same concentration, verified the method's repeatability. The RSD as a percentage was computed. The method's intermediate precision was verified using intra- and inter-day analysis, which is the examination of the formulation was carried out three times on three consecutive days and three times within the same day at one-hour intervals. Both the medication quantity and the percentage RSD were computed[11-13]. The findings of precision studies conducted within and between days are presented in (Table 3 and 4)

Ruggedness Study

It conveys the accuracy of laboratory variances such as various analysts. The method's robustness was evaluated three times using the same equipment by a different analyst[14,15]. The outcome was displayed in (Table 5) as a percentage RSD.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Following the standardization curve, determine the LOQ and LOD independently. The LOD and LOQ were determined with the help of a regression line's residual standard deviation or its standard deviation of regression line y-intercepts. The LOQ and LOD values were calculated using mean of response's slope and standard deviation (intercept). Calibration standards were used to calculate the LOD and LOQ of adapalene and quercetin using the recommended procedures.

LOD = 3.3σ/S and LOQ = 10σ/S, σ is the response standard deviation (intercept) and slope of the calibration curve S[16-18]. (Table 1) displays the LOD and LOQ results.

RESULTS AND DISCUSSION

The solutions of 10μg/ml for both Adapalene and Quercetin were analyzed and the λmax the observed values 320 nm and 370 nm, respectively. The calibration curve of Adapalene and Quercetin at 320nm and 370nm were plotted (Figures 3, 4). The relationship between the absorbance and the concentration of Adapalene and Quercetin It has linear

in the range of 2-10 μg/mL at both wavelengths 320 nm and 370 nm. The regression coefficient for Adapalene and Quercetin has been found to be linear 0.9989 and 0.9983 respectively which specifies good association between concentration and absorbance within the concentration range tested. The limit of detection of Adapalene and Quercetin has been found to be linear 0.111μg/ml and 3.40μg/ml respectively. Where, the limit of quantification for Adapalene and Quercetin It has been found to be linear 0.33μg/ml and 3.16 μg/ml. Evaluation Interday and Intraday precision It has been found to be lineless than two (<2), This indicates good precision.

CONCLUSION

In compliance with the ICH recommendations, a method for concurrently determining quercetin and adapalene was developed and verified. According to the evaluation results, the suggested UV spectroscopy method offers an easy, quick, accurate, and exact analytical method.

Acknowledgement

The authors are thankful to Glenmark pharmaceuticals Pvt. Ltd Nashik for providing the gift sample of Adapalene.

Abberivations

DMSO: Dimethyl sulfoxide; **ICH**: International Conference of Harmonization; **API**: Active pharmaceutical ingredient; **LOD:** Limit of Detection**; LOQ:** Limit of Quantitation

REFERENCES

1. Ahmad Nasrollahi S, Koohestani F, Naeimifar A et al. Preparation and Evaluation of Adapalene Nanostructured Lipid Carriers for Targeted Drug Delivery in Acne. Dermatologic Therapy. 2021;34(2):e14777. DOI:10.1111/dth.14777
2. Halagali P, Wannur VI, Kumar AKA et al. Formulation and Evaluation of Quercetin Ethosomal Hydrogel for Topical Delivery System. International Journal of Pharmaceutical Investigation. 2024;14(3):749-58. DOI: 11.2.1
3. Sahoo NG, Kakran M, Shaal LA, et al. Preparation and Characterization of Quercetin Nanocrystals. Journal of Pharmaceutical Sciences. 2011;100(6):2379-90. DOI:10.1002/jps.22446
4. Kamal AH, El-Malla SF, Hammad SF. A Review on UV Spectrophotometric Methods for Simultaneous Multicomponent Analysis. European Journal of Pharmaceutical and Medical Research. 2016;3(2):348-60
5. Ravindra K. Simultaneous Estimation and Statistical Evaluation of Developed Validated Methods for Combined Drugs in Marketed Formulation. Journal of Pharmaceutical Research. 2013;12(1):23-9
6. Gupta D, Bhardwaj S, Sethi S et al. Simultaneous Spectrophotometric Determination of Drug Components From Their Dosage Formulations. Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy. 2022;270:120819. DOI:10.1016/j.saa.2021.120819
7. Vyas AJ, Jha SA, Patel AB. Review on Simultaneous Equation Method (Vierodt's Method). Asian Journal of Pharmaceutical Analysis. 2022;12(2):149-56. DOI:10.52711/2231-5671.2022.00026
8. Korany MA, Wahbi AM, Mandour S, Elsayed MA. Determination of Certain Drugs in Multicomponent Formulations by First Derivative Ultraviolet Spectrophotometry. Analytical Letters. 1985;18(1):21-34. DOI:10.1080/00032718508066922
9. Stenlake JB. Practical pharmaceutical chemistry. London: Athlone Press; 1975
10. Rao CR, Shinozakt N. Precision of Individual Estimators in Simultaneous Estimation of Parameters. Biometrika. 1978;65(1):23-30. DOI:10.1093/biomet/65.1.23
11. Sen AK, Sen DB, Maheshwari RA, Balaraman R, Seth A. Simultaneous Estimation of Aliskiren Hemifumarate and Hydrochlorothiazide in Combined Tablet Formulation by Simultaneous Equation, Absorbance Ratio and First Derivative Spectroscopic Methods. Journal of Applied Pharmaceutical Science. 2016;6(7):164-70. DOI:10.7324/JAPS.2016.60724
12. Dhartarkar PG, Kalamkar RV, Suprit D. Development and Validation of UV Spectrophotometric Method for Estimation of Dexibuprofen in Bulk and Dosage Form. Scholars Res Library Pharmaceutical Chem. 2011;3(4):361-6
13. Singh U, Baldi A. Simultaneous Estimation of Quercetin and Silymarin: Method Development and Validation. International Journal of Pharmaceutical and Biological Archives. 2013;4(3):527-31
14. Chaudhari S, Mannan A, Daswadkar S. Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Acyclovir and Silymarin in Niosome Formulation. Scholar Research Library. 2016;8(5):128-33
15. Khanage SG, Mohite PB, Jadhav S. Development and Validation of UV-Visible Spectrophotometric Method for Simultaneous Determination of Eperisone and Paracetamol in Solid Dosage Form. Advanced Pharmaceutical Bulletin. 2013;3(2):447-51. DOI:10.5681/apb.2013.073
16. Gholse YN, Chaple DR, Kasliwal RH. Development and Validation of Novel Analytical Simultaneous Estimation-Based UV Spectrophotometric Method for Doxycycline and Levofloxacin Determination. Bio Interface Research in Applied Chemistry. 2022;12(4):5458. DOI:10.33263/BRIAC12454585478
17. Celia C, Di Marzio L, Locatelli M et al. Current Trends in Simultaneous Determination of Co-administered Drugs. Separations. 2020;7(2):29. DOI:10.3390/separations7020029
18. Chaudhary J, Jain A, Saini V. Analytical Method Development and Validation for the Simultaneous Estimation of Alprazolam and Propranolol in Their Combined Dosage Form. International Journal of Drug Delivery. 2012;4(3):310-5