Green analytical methods in practice: chemometrics driven methods for the simultaneous estimation of darunavir and ritonavir

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Abstract

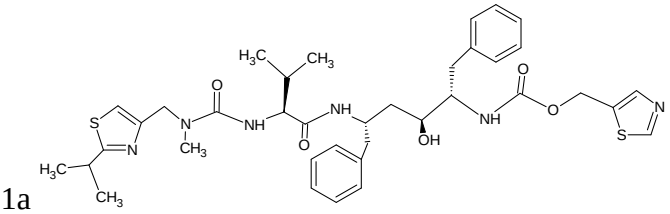
A fixed-dose combination of Darunavir and Ritonavir is effective for the treatment of human immunodeficiency virus (HIV) infection. For the simultaneous estimation of the fixed-dose combination, two chemometrics methods are developed based on ratio mean centring (RMC method) and the difference between the adjacent data points (DBADP method). The methods employ spectral ratio manipulation, wherein the ratio spectral data is subjected to mean centring in the RMC method, and the difference between the adjacent data points in the ratio spectra are used for the DBADP method. For the data processing, a simple software program written with Python and MATPLOTLIB was used. Beer's law was valid in the range of 10-60 µg/ml for Darunavir and 2.5-15 µg/ml for Ritonavir in both methods. The assay results obtained for the marketed formulation were found to be 99.78% and 99.36% for Darunavir and Ritonavir, respectively, by the RMC method: Assay results were 99.91% and 100.76% for Darunavir and Ritonavir respectively by DBADP method.

Keywords: chemometrics, Darunavir, Ritonavir, RMC, DBADP

1.Introduction

Darunavir ethanolate (DRV) is chemically [(1S,2R)-3-[[(4-aminophenyl)sulfonyl] (2-methylpropyl)amino)-2-hydroxy-1-(phenylmethyl) propyl] carbamic acid (3R,3aS,6a-R)-hexahydrofuro [2,3-b] furan-3-yl ester monoethanolate. [1] It is a protease inhibitor (PI) and is effective in human immunodeficiency virus (HIV) type-1 infection. Ritonavir (RIT) is chemically (5S,8S,10S,11S)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-

dioxo8,11bis (phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid-5-thiazolylmethyl ester [1]. It is a selective, competitive and reversible inhibitor of both HIV-1 and HIV-2 proteases. It is mostly used in the treatment of AIDS and particularly to inhibit the liver enzyme, viz., cytochrome P450-3A4 (CYP3A) [3]. The chemical structure of Darunavir and Ritonavir is given in Fig Ia and b.



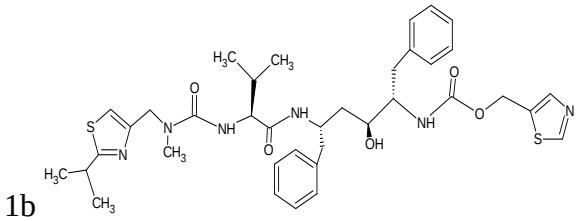


Figure 1a) Chemical structure of Darunavir b)Ritonavir

Darunavir, co-administered with Ritonavir or with other antiretroviral agents, is effective for the treatment of human immunodeficiency virus (HIV) infection.

An exhaustive survey of literature has shown the presence of some analytical methods for Darunavir and Ritonavir either alone or in fixed-dose combinations with each other or other drugs. Darunavir is analysed by UV spectrophotometry,3-55 and LC methods6-20, and Ritonavir is studied by UV spectrophotometry,21-23 and LC methods24-38. The fixed-dose combination of Darunavir and Ritonavir is studied by UV spectrophotometry39,40 LC41-50 and HPTLC methods.51 Though there are several methods available for the fixed-dose combination of Darunavir and Ritonavir, the UV spectrophotometric methods are meagre, and hence we have developed two new strategies based on chemometrics for the simultaneous estimation of the same.

There are several reported analytical studies available in the literature that uses chemometrics for the simultaneous analysis of binary and ternary samples.52-55. In this manuscript, two chemometrics methods are developed, namely, the difference between the adjacent data point (DBADP) method and the ratio mean centring (RMC) method. In chemometrics methods, helpful information can be extracted using statistical or mathematical tools to facilitate the QC analysis.

Compared to other analytical methods, spectrophotometric methods are preferable due to cost consideration and ease of handling of the instrument. As binary or ternary mixtures cannot be analysed by simple spectrophotometric methods, chemometrics methods are a good option. In the developed methods, the use of an organic solvent is maximum avoided, and hence the methods are termed the green method.

2.Experimental

2.1 Instruments and chemicals

A double-beam UV-visible spectrophotometer (Shimadzu, 1800) with UV-probe software was used for primary data acquisition and later for ratio spectra recording. For chemometrics methods (DBADP and RMC), a simple program was written using Python and Matplotlib. Matched and calibrated quartz cuvettes were used as sample cells. Hetero Drugs Ltd, Hyderabad, India, provided DRV and RIT com

2.2 Analytical method development and validation

Methodology for RMC and DBADP

Ratio spectra are obtained by dividing the zero-order spectrum of each drug by a suitable concentration of the second drug as the divisor. The RMC method depends on the mean centring of the data points in the ratio spectra, whereas the difference between the adjacent points of the ratio spectra is used for the DBADP method. Both methods help estimate drugs in binary or ternary mixtures, which precludes any extraction or separation process. The minimum or maximum values obtained as amplitudes in the manipulated ratio spectra concerning wavelength was used to construct the calibration graphs.

2.4 Method validation

The method was validated for accuracy, precision, linearity, LOD, and LOQ as per ICH guidelines[56] the detailed procedure of which is given below.

2.4.1 Linearity

Ten mg of each standard drug was dissolved separately in 1 ml of methanol and then 0.1N SLS to obtain stock solution (1000 µg/ml) of each drug. These solutions were diluted suitably with 0.1N SLS to obtain the standard DRV (10–60 µg/ml) and RIT (2.5–15 µg/ml) of various concentrations. Standard solutions were scanned in the UV range. The ratio spectra of DRV solutions were prepared by dividing each zero-order spectra by ten µg/ml of RIT spectra. The ratio spectra of RIT solutions were prepared by dividing each zero-order spectra with 15 µg/ml of DRV spectra. The data points corresponding to the ratio spectra were collected and were subjected to ratio mean centring and difference between adjacent data points using a simple program written for this purpose. For both methods, calibration graphs were constructed by plotting the amplitudes versus corresponding concentrations.

2.4.2 Accuracy

The standard addition process assessed the accuracy by means of the closeness between the actual and the value found. Standards were spiked at three levels (80%, 100% and 120%) to commercially available tablets in triplicate. The method's accuracy and reproducibility are proved by calculating the amount of drug recovered and the values of % relative standard deviation (RSD) that should be <2.0 by both methods.

2.4.3 Precision

The precision assessment measures the closeness of test results upon multiple sampling of the homogenous sample. The method repeatability (intra-day precision) was determined by the triplicate analysis of three standard solutions of DRV (10, 20, 30 µg/ml) and RIT (5, 10, 15 µg/ml) of concentrations for each drug. Inter-day (n=3) analysis of the same concentration of DRV and RIT was used for Intermediate precision. The statistical analysis of precision data proved good precision of the methods as the % RSD was <2.0 for both drugs in interday and intraday precision studies.

2.4.4 Limit of detection and limit of quantitation

Limit of detection and limit of quantitation

We used samples containing low analyte concentrations to determine the detection limit (LOD) and quantitation limit(LOQ). Formulae 1 and 2 are used for calculating LOD and LOQ.

$LOD = 3.3\ \sigma/S$ Formula 1

$LOQ = 10\ \sigma/S$ Formula 2

S = slope of the calibration curve and σ = standard deviation of the response

2.4.5 Analysis of the pharmaceutical dosage form

The marketed fixed-dose formulation of DRV and RIT tablets are available in two strengths: DANAVIR R (Darunavir: 800 mg. Ritonavir : 100 mg) and DURART R 450 (Darunavir: 400 mg Ritonavir: 50 mg). Twenty tablets of each strength were powdered. A quantity equivalent to 10 mg of DRV was taken in a 10 ml volumetric flask, added 1 mL of methanol and dissolved and further diluted with 0.1N SLS with the help of sonication for 15 min. The solutions were filtered. An aliquot was diluted to get a sample solution of 20 µg/ml of DRV and 2.5 µg/ml of RIT for each strength. For the preparation of ratio spectra, the formulation's spectra were divided by the spectra of each drug one by one. The amplitude values corresponding to 230 and 274 nm were considered for the RMC method. For the DBADP method, the amplitudes were measured at 224 and 273 nm for DRV and RIT, respectively, to assess drug content. The substitution of the amplitudes into the straight-line equation yields the content of DRV and RIT by both methods.

3.Results and Discussion

3.1 Method optimisation

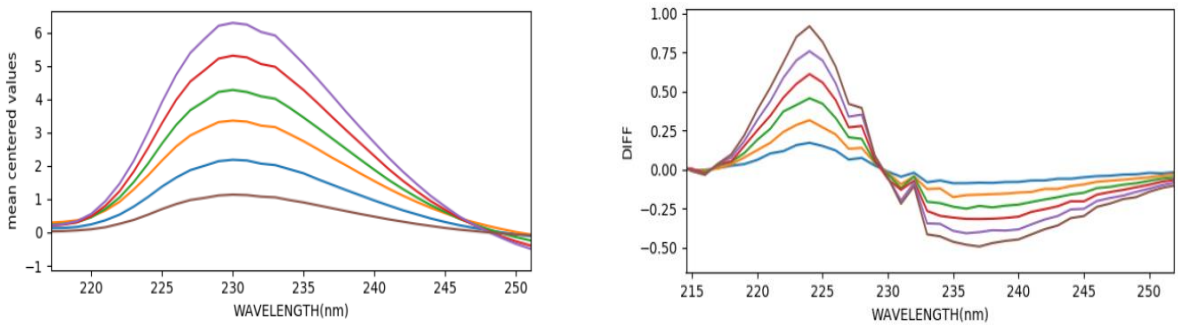
The methods depend on the spectral ratio manipulation wherein the selection of divisor concentration was critical in getting minimum or maximum signal in the RMC graph and DBADP graph of each drug. Hence, for RIT, the divisor was selected as ten µg/mL of DRV; and for DRV, it was 12.5 µg/mL of RIT. Δλ was chosen as 1.0 nm and did not affect the signal.

3.2 Calibration plot for DRV and RIT

DRV and RIT showed a linear relationship between concentration (µg/ml) and the corresponding amplitudes in RMC and DBADP methods. By both methods, DRV and RIT were linear in the range of 10–60 µg/ml and 2.5–15 µg/ml, respectively. By linear regression analysis, the correlation coefficient value (R^2) for DRV and RIT was calculated by both methods. Figures 2 and 3 represent the RMC spectra and the DBADP spectra for DRV and RIT, respectively, which indicate the linear relationship between the drug concentration and the corresponding signals. The details of analytical characteristics are given in Table 1.

Table 1: Analytical characteristics for DRV and RIT by RMC and DBADP methods

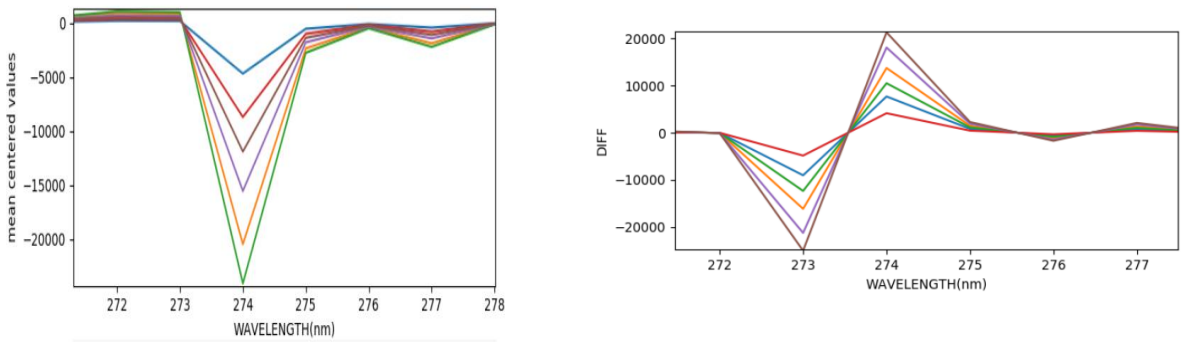
Method	Drug	λ(nm)	Linearity range (µg/mL)	Regression equation, R^2
RMC	DRV	230	10–60	f(x)=0.1031x+0.1508, 0.999
	RIT	274	2.5–15	f(x)=-1607.8x-601.99, 0.9995
DBADP	DRV	224	10–60	f(x)=-0.0149x-601.99, 0.9994
	RIT	273	2.5–15	f(x)=-1621x-429.78, 0.999



A B

Fig Overlay ratio spectra of DAR (10 to 60 µg/mL) by RMC method (A) DBADP method (B)

A B



A.Overlay ratio spectra of RIT (2.5 to 15 µg/mL) by RMC method (A) DBADP method (B)

3.3 Accuracy

The drugs' percentage recoveries were computed from the corresponding regression equations obtained in the linearity studies in the RMC and DBADP methods. Drug concentrations of DRV and RIT present in the spiked samples were calculated by the RMC method and tabulated in Table 2. The % recoveries of DRV and RIT were obtained in the range of 99.59 – 100.69 and 100.22 - 102.26, respectively, by the RMC method. The percentage recoveries of the drugs by the DBADP method were calculated and tabulated in Table 3. The % recoveries of DRV and RITwere obtained in the range of 99.21 – 99.88 and 99.72 - 102.51, respectively, by the DBADP method. The %RSD values were found to be <2.0 in both ways.

Table 2. Recovery data by RMC method

Spiking Level (%)	Drug	Actual con. (µg/ml)	Con. recovered (µg/ml)* AM±SD (n=3)	Recovery (%)	%RSD
80	DRV	36	36.09±0.15	100.25	0.438
	RIT	4.5	4.45±0.06	99.08	1.392
100	DRV	40	40.62 ±0.64	101.55	1.582
	RIT	5.0	5.11±0.05	102.26	1.11

120	DRV	44	43.82±0.51	99.59	1.164
	RIT	5.5	5.54±0.04	100.76	0.773

*Acceptance Criteria: % RSD should not be more than 2

Table 3. Recovery data by DBADP method

Spiking Level (%)	Drug	Actual con. (µg/ml)	Con. recovered (µg/ml)* AM±SD (n=3)	Recovery (%)	%RSD
80	DRV	36	36.10±0.21	100.28	0.607
	RIT	4.5	4.54±0.06	100.88	1.37
100	DRV	40	40.54 ±0.51	101.36	1.273
	RIT	5.0	5.11±0.05	102.26	0.97
120	DRV	44	43.77±0.45	99.47	1.04
	RIT	5.5	5.57±0.67	101.38	1.21

3.4 Precision

The method's repeatability (intra-day precision) was determined by inter-day (*n*=3) analysis of three of the selected standard solutions of DRV and RIT at the concentration of 2, 6 and 10 µg/mL. The intra-day precision data is represented in Table 5. The inter-day precision analysis was also performed at the same concentration levels but on different days, and data are reported in Table 6. The statistical analysis of precision data proved good precision of the methods as the % RSD was <2.0 for both drugs in interday and intraday precision studies.

Table 5. Precision data by RMC methodology

Drug name	Actual Conc. (µg/mL)	Intra-day precision		Inter-day precision	
		Conc. Found (µg/mL) (AM ± SD) (n=3)	% RSD	Conc. Found (µg/mL) (AM ± SD) (n=3)	% RSD
DRV	10	9.80±0.08	0.81	9.78±0.14	1.53
	20	19.160±0.08	0.90	20.39±0.03	0.14
	30	30.460±0.06	0.21	29.77±0.17	0.57
RIT	5	5.06±0.07	0.81	4.98±0.07	1.40
	10	10.30±0.06	0.90	10.38±0.20	1.92
	15	14.42±0.11	0.21	14.71±0.07	0.48

*Acceptance Criteria: % RSD should not be more than 2

Table 6. Precision data by DBADP methodology

Drug name	Actual Conc. (µg/mL)	Intra-day precision		Inter-day precision	
		Conc. Found (µg/mL) (AM ± SD) (n=3)	% RSD	Conc. Found (µg/mL) (AM ± SD) (n=3)	% RSD
DRV	10	9.91±0.06	0.66	9.83±0.06	0.67
	20	19.89±0.30	1.53	19.80±0.08	0.42
	30	29.45±0.10	0.36	29.88±0.04	0.15
RIT	5	5.07±0.05	1.01	4.89±0.08	1.63
	10	9.99±0.18	1.81	10.36±0.13	1.29
	15	14.75±0.12	0.83	15.11±0.07	0.46

*Acceptance Criteria: % RSD should not be more than 2

3.5 Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of DRV and RIT were calculated by both RMC and DBADP methods and the values are tabulated in table 5.

Table 4. LOD and LOQ values

Method	DRV (µg/ml)		RIT (µg/ml)	
	LOD	LOQ	LOD	LOQ
RMC	0.03	0.09	0.02	0.06
DBADP	0.66	1.98	0.01	0.03

3.6 Analysis of commercial tablets (assay)

The accuracy of the proposed methods were evaluated by the assay of commercially available tablets Danavir R(DRV (800 mg) and RIT (100 mg)) and DURART R 450 **(**DRV (400 mg) and RIT (50 mg)). The results obtained for DRV and RIT were compared with the corresponding labeled amounts and reported in Table 6. The % RSD for assay results of the formulation was <2, which indicated the accuracy of the proposed method.

Table 6: Assay results of commercial tablets

Formulation with label claim	Amount found in mg (AM) ± SD; RSD(%) (n=3)			
Danvir R, DRV=800 mg, RIT=100 mg	RMC		DBADP	
	DRV	RIT	DRV	RIT
	798.3±0.98,0.12	99.36±0.30,0.30	799.26±0.95 ,0.12	100.76±0.47, 0.46

% Assay	99.78	99.36	99.91	100.76
Durart R 450, DRV=400 mg, RIT=50 mg	398.15±0.42,0.11	49.36±0.28,0.57	408.63±0.51,0.12	50.62±0.36, 0.71
% Assay	99.54	98.72	102.15	101.24

4.Conclusion

The purpose of the research work was to develop an economical and eco-friendly analytical method based on chemometrics-assisted UV spectroscopy for the simultaneous analysis of DRV and RIT in the tablet dosage form. The developed method does not require any sophisticated instruments like HPLC or HPTLC nor costly reagents or solvents. Since two chemometrics methods are used without any derivative steps, maximum information is available without loss of signal-noise ratio.

Competing Interests

The authors declare no conflict of interest.

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