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Relative Response Factor for Lamivudine and Zidovudine Related substances by RP-HPLC with DAD detection

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Abstract

A study was conducted to establish the Relative Response Factors for Zidovudine related impurity C, Lamivudine salicylic acid and Zidovudine related impurity B. A simple and fast isocratic Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was used for the simultaneous determination of the related impurities. The method consists of a mobile phase combination of Acetonitrile (HPLC grade) and Buffer (0.0680 g of Potassium Dihydrogen Orthophosphate, 0.3 ml of Triethylamine, pH adjusted to 8.0 with Orthophosphoric acid to a final volume preparation of 100 ml) in the ratio 10:90 using Phenomenex Luna 5-µm C18 (2)-250 x 4.6-mm, 5-µm) as a stationary phase, flow rate of 1.0 mL/min with detection at 270 nm. The RRF for Zidovudine related impurity C, Lamivudine salicylic acid and Zidovudine related impurity B were 2.07, 0.13 and 1.28 respectively. Results obtained for quantification of the related substance in lamivudine and zidovudine single dose oral solid dosage form using the RRF and know standards shows no significant difference at 95 % confidence interval. The RRF can therefore be used for the quantification of know related impurities in lamivudine zidovudine oral dosage form using the stated chromatographic conditions.

1. INTRODUCTION

Relative Response Factor (RRF) is an analytical parameter used in chromatographic procedures to control impurities/degradants in drug substance and drug product. RRF is used to correct the difference in detector response of impurities with analyte peak. RRF is established by slope method with linear range of solutions.

As per United States Pharmacopoeia (USP) the Relative response factor, is the ratio of the responses of equal amounts of the Impurities and the drug substance [1]. It is referred as Correction factor, response factor or relative response factor in the USP.

European Pharmacopoeia refers RRF as correction factor or response factor. The correction factor is reciprocal of the response factor. Response Factor is expressed as the sensitivity of a detector for a given substance relative to a standard Substance [2].

As per British Pharmacopoeia (BP) RRF is expressed as Response Factor. The Response Factor is a relative term, being the response of equal weights of one substance relative to that of another in the conditions described in the test [3].

RRF are convenience for usage as long as the stated chromatographic condition has been followed with no change in parameters. RRF are necessary in impurity testing due to high cost of impurity standards, stability of the standards and difficulty in the isolation of this standards for usage. RRF are used in drug purity testing, in limit tests, in mass balance test etc.

The aim of this study is to establish the Relative Response Factors for Zidovudine related impurity C, Lamivudine salicylic acid and Zidovudine related impurity B and to compare the recovery using the RRF and know standards in lamivudine zidovudine single oral dosage form.

2. MATERIALS AND METHODS

2.1 Materials and Reagents:

Chemicals / Reagents: Acetonitrile (Manufacturer: Fisher Scientific, Batch #: 0803950), USP Purified Water and Doubly distilled water.

Analytical Reference Standards: USP Lamivudine (Lot #: H0H087, Potency: 99.6 %), USP Zidovudine RS (Lot #: HOF263, Potency: 99.0 %), USP Zidovudine Related Compound B RS (Lot #: H0F230, Potency: 100.0 %), USP Zidovudine Related Compound C RS (Lot #: GOG181, Potency: 100.0 %), Salicylic acid (Lot #: KOF112),

Potency: 99.8 %). Lamivudine (Manufacturer: Shanghai Desano Chemical Co. LTD. China, Lot #: DH010-4-090405, Potency (OAB): 98.34 %. Zidovudine (Manufacturer: Shanghia Desano Chemical CO Ltd, Lot #: DH006-4-090430, Potency (OAB): 100.93 %.

Pharmaceutical Excipients: Microcrystalline cellulose (Batch #: 0028, Manufacturer: Brahmar Cellulose Products PVT Ltd, Solutab (Croscamellose Sodium) (Batch #: 8199/09, Manufacturer: Blanver Farmoquimica Ltd, Magnesium Stereate (Batch #: MGSV80097, Manufacturer: Stockbridge International Ltd. Aerosil (Colloidal Silicon Dioxide) (Batch #: 132700021, Manufacturer: Biochemie.

2.2 Instrumentation and Chromatographic conditions.

Agilent Technologies 1200 series HPLC modules (G1315D, G1315A, G1329A, G1311A, G1332A and organizer, with ChemStation data processing software), Sonicator, OHAUS Analytical Balance. Stainless steel column 250 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (Phenomenex Luna 5-µm C18 (2)-250 x 4.6-mm, 5-µm). Mobile phase composition was 90(buffer): 10 Acetonitrile. Buffer was prepared from 0.0680 g of Potassium Dihydrogen Orthophosphate, 0.3 ml of Triethylamine, pH adjusted to 8.0 with Orthophosphoric acid diluted with distilled water to a final volume of 100 ml. The mobile phase was pumped through the column at a flow rate of 1 mL/minute. The sample injection volume was 10 µL. The DAD detector was set at a wavelength of 270 nm for the detection. Column oven was set at 30±1 ^{o}C and the run time was 25 minutes.

2.3 Calibration curve for Relative Response Factors (RRF).

Linearity and Working Concentration range, LOD and LOQ were determine for Zidovudine related impurity C, Lamivudine, salicylic acid, Zidovudine and Zidovudine related impurity B by two different analyst.

A graph of concentration versus response for impurity solutions and standards were plotted. (Concentrations of impurity solutions on 'X' axis and response or area on 'Y' axis). The equation of the calibration curve and correlation coefficient were determined. The relative response factor were calculated by both analyst one and analyst two.

Formula for Calculation

Relative response factor of impurity = [Slope of impurity solution in curve/ Slope of standard solution in curve]

The precisions were determined using the RRF by evaluating intraday precision and interday precision by preparation and analysis of the same sample (all known standards and placebo) in six replicates. This was evaluated at various time points (0 (initial), 4, 8, 12, 16, 20 and 24 hours) by the same analyst. Interday precision was performed in six replicates at intervals of one day by two different analysts over a period of six days. The Percentage contents and relative standard deviation (RSD) were determined in each case. The results were subjected to statistical analysis at 95 % confidence interval to determine any significant differences.

Accuracy was determined using method of spiking. RRF and known impurity Standard concentration were used to estimate the impurity in the sample. Six different amounts corresponding to 80 %, 100 % and 120 % concentrations of zidovudine related impurity C, Lamivudine, Lamivudine salicylic acid, Zidovudine, and Zidovudine related impurity B working standard and placebo were analysed. The nominal concentrations were compared to the actual concentrations using RRF and known standards. The percentage recoveries were noted and statistical analysis at 95% confidence interval to determine any significant differences.

2.5 Application of RRF for the Analysis of Tablet Formulation

Twenty tablets were weighed, the average weight was noted and powdered. Samples of the powdered tablet equivalent to 48 mg of lamivudine and 98 mg of zidovudine were weighed and transferred into a 100 mL volumetric flask. 60 mL of the diluent was added and sample sonicated for 5 minutes. Solution was made to volume with the diluent.

Approximaly 0.0124 mg/mL of Zidovudine related compound C, lamivudine, lamivudine salicylic acid, zidovudine and Zidovudine related compound B were used for the quantification of the related impurities. Recovery using both RRF and Standards were compared using ANOVA at 0.05 significant level.

3. RESULTS AND DISCUSSION

To develop a relatively simple system, Acetonitrile (HPLC grade) and Triethylamine were used as the only organic solvent with buffer (Buffer (0.0680 g of Potassium Dihydrogen Orthophosphate, 0.3 ml of Triethylamine, pH adjusted to 8.0 with Orthophosphoric acid to a final volume preparation of 100 ml)

Various mobile phase acetonitrile to buffer in the ratios 40:60, 20:80 and 10:90 were used. Mobile composition which gave better resolution was observed to be 10(Acetonitrile): 90 (buffer).

Phenomenex Luna 5-µm C18 (2)-(250 x 4.6-mm, 5-µm) as the stationary phase was adopted and used for the analysis. A wave length of 270 nm was adopted for appreciable peak area for the analyte. A flow rate of 1mL/min, run time of 25 minutes, injection volume of 10 µL and a column oven temperature of 30 ^{o}C were adopted for the analysis.

3.1 Establishment of relative response factor

Linearity of detector response

Linearity and Working Concentration range, LOD and LOQ were determine for Zidovudine related impurity C, Lamivudine, Lamivudine salicylic acid, Zidovudine and Zidovudine related impurity B by two different analyst. The working concentration ranges from 0.0081 mg/mL to 0.0209mg/mL for Zidovudine related impurity C, 0.0082 mg/mL to 0.0207 mg/mL for Lamivudine, 0.0085 mg/mL to 0.0206 mg/mL for Lamivudine salicylic acid, 0.0081 mg/mL to 0.0205 mg/mL for zidovudine and 0.0086 mg/mL to 0.0207 mg/mL for Zidovudine related impurity B. The Correlation co-efficient were observed to be in the range of 0.9975 to 0.9999 respectively. With regards to residual plot, the residuals were randomly dispersed.

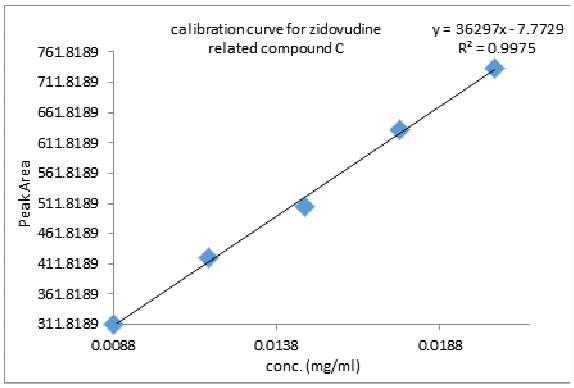

Fig. 1: Calibration curve for for zidovudine related compound C

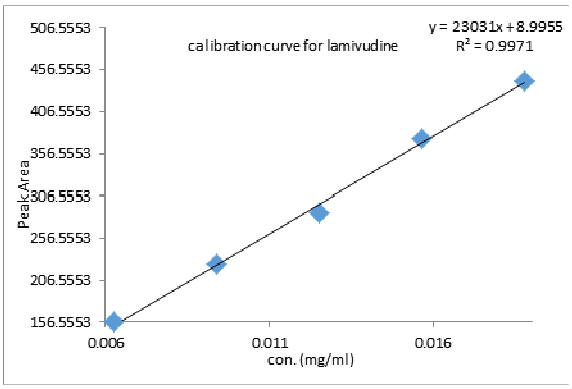

Fig. 2: Calibration curve for Lamivudine

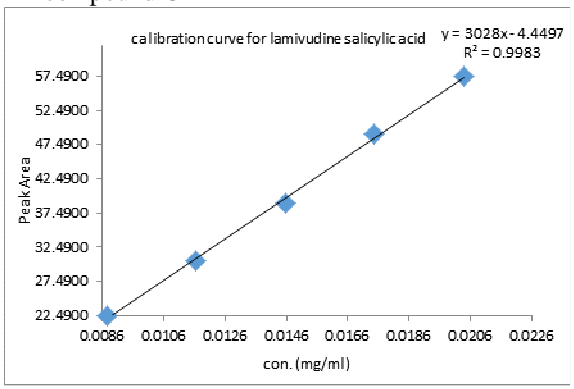

Fig 3: Calibration curve for lamivudine salicylic acid

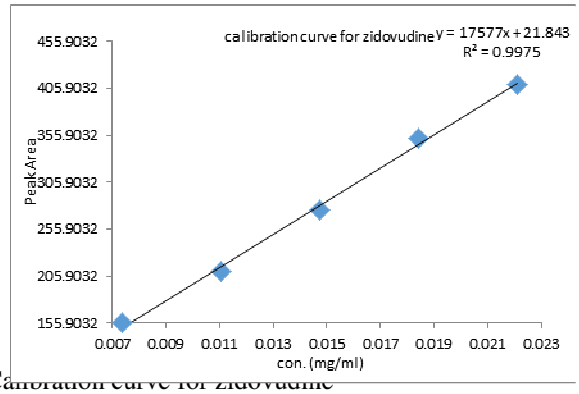

Fig 4:Calibration curve for zidovudine

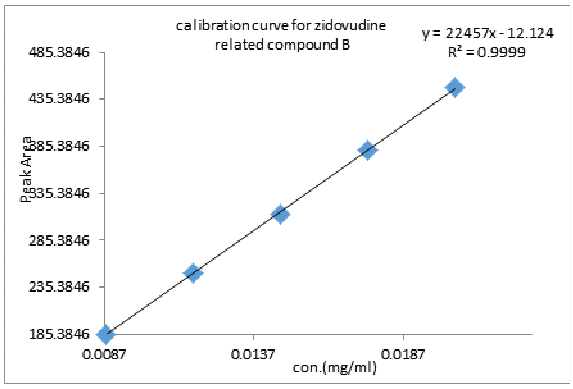

Fig 5: Calibration curve for Zidovudine related compound B

Table 1: Linear regression data, LOQ, LOD and RRF for calibration curves by analyst one

API	Zidovudine related compound C	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
Concentration range (mg/mL)	0.0088to 0.0204	0.0082 to 0.0.0204	0.0087 to 0.0204	0.0082 to 0.0205	0.0088 to 0.0204
slope	36297	23031	3028	17577	22457
Intercept	-7.7729	8.9955	-4.4497	21.843	-12.124
Correlation coefficient	0.9975	0.9971	0.9983	0.9975	0.9999
LOD	1.12E-04	1.72E-04	1.44E-03	2.88E-04	1.67E-04
LOQ	3.40E-04	5.21E-04	4.36E-03	8.73E-04	5.07E-04
RRF	2.07		0.13		1.28

Table 2: Linear regression data, LOQ, LOD and RRF for calibration curves by analyst two

API	Zidovudine related compound C	Lamivudine	Lamivudine Salicylic acid	Zidovudine	Zidovudine related compound B
Concentration range (mg/mL)	0.0081 to 0.0209	0.0083 to 0.0.0207	0.0085 to 0.0206	0.0081 to 0.0205	0.0086 to 0.0207
slope	36302	23042	3054	17534	22489
Intercept	-6.0032	95	-5.6756	12.434	-12.565
Correlation coefficient	0.9977	0.9997	0.9983	0.9991	0.9999
LOD	1.12E-04	1.72E-04	1.43E-03	2.89E-04	1.67E-04
LOQ	3.40E-04	5.21E-04	4.32E-03	8.75E-04	5.06E-04
RRF	2.07		0.13		1.28

The % RSD obtained for Intraday and interday precision using RRF for the quantification of related impurities were in the range of 0.14 – 0.36 and 0.25 to 0.65 respectively. The recovery has a high precision since % RSD were less than 1.00 %. Analysis of the variance in the recovery both for interday and intraday precision reveals no statistically reliable difference since P value obtained were all less than 0.05.

Table 3: Recovery for Intraday precision over study period of 24 Hours

	Zidovudine related compound c	Lamivudine Salicylic acid	Zidovudine related compound B
Mean Recovery (%)	100.34±0.14	99.78±0.36	99.94±0.26
% RSD	0.14	0.36	0.26

Table 4: Recovery for interday precision by First analyst over a period of Six days.

	Zidovudine related compound c	Lamivudine Salicylic acid	Zidovudine related compound B
Mean Recovery (%)	99.91±0.25	99.98±0.53	99.94±0.65
% RSD	0.25	0.53	0.65

The accuracy of the recovery was determined at three different concentration levels of placebo missed with standards. The percentage recovery were found to be in the range of 98.78 % - 100.21 %. Analysis of variance performed at 95 % confidence interval reveals that there were no significant differences between the results obtained at the various concentration levels. P value observed were greater than 0.05, ($p > 0.05$).

Table 6: Mean Recovery at different concentration level.

Mean Recovery	Zidovudine related compound c	Lamivudine Salicylic acid	Zidovudine related compound B
80%	99.90±0.17	99.65±0.53	100.07±0.56
100%	99.99±0.64	99.66±0.64	99.70±0.11
120%	100.21±0.35	98.78.03±0.12	99.78±0.21

The mean recovery of Zidovudine related compound c, Lamivudine Salicylic acid, Zidovudine and Zidovudine related compound B in zidovudine lamivudine single oral solid dosage in six replicates were quantified using the RRF and known standards. Refer to table 7 and table 8 for percentage recovery. Analysis of variance performed at 95 % confidence interval reveals that there were no significant differences between the results obtained at the various concentration levels. P value observed were greater than 0.05, ($p > 0.05$).

Table 7: Mean Percentage of known related impurities in tablet using RRF.

Zidovudine related compound c (%)	Lamivudine Salicylic acid (%)	Zidovudine related compound B (%)
0.0124±0.000125	0.0034±0.00013	0.0046 ± 0.000021

Table 8: Mean Percentage of known related impurities in tablet using know impurity standards.

Zidovudine related compound c (%)	Lamivudine Salicylic acid (%)	Zidovudine related compound B (%)
0.0123±0.000133	0.00341±0.00014	0.0045 ± 0.000022

4. CONCLUSION

Relative Response Factor (RRF) is a common analytical parameter frequently used in many chromatographic procedures for quantitative or limit tests for impurities because in many cases the corresponding reference standards are not available.

The RRF calculated for Zidovudine related compound c, Lamivudine Salicylic acid, and Zidovudine related compound B were observed to be 2.07, 0.13 and 1.28 respectively. Recovery of impurity mixed with placebo and in tablet using both RRF and standards show no significant difference at 95 % confidence interval. The RRF calculated can therefore be used to quantify known related substances in lamivudine zidovudine single oral solid dosage form.

Reference:

1. United States Pharmacopeia USP 34-NF 29, Chapter, 621, Rockville (2011)
2. European Pharmacopoeia 7.0, Section 2.2.46 Chromatographic Separation Techniques, (2010).
3. British Pharmacopoeia, Control of Impurities, Supplementary Chapter I, SC1 A, (2009).

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