

CHAPTER 8

Methodological Approaches for the Simultaneous *In Vitro* Analysis of Rifampicin and Isoniazid

8.1. Introduction

The RIF-INH combination is instituted in the majority of commercially available FDCs for the chemotherapeutic management of TB during the intensive and continuation phases. Dissolution testing with subsequent analysis is considered as an imperative tool for quality evaluation of the combination, however the degree of disparity often encountered between dissolution testing and bioavailability cannot be ignored, and due consideration must be given to the *in vitro* testing conditions of the combination (Acocella et al., 1988).

The crux of this research was the counteraction of the reduction in RIF bioavailability on coadministration with INH, under stomach acid conditions, as replicated by dissolution studies in USP-recommended media, which otherwise culminates in accelerated RIF degradation. This bioavailability reduction has also been considerably reported when the drug is combined with the principal anti-TB agents in single oral solid formulations. Thus, apart from rate and extent of drug release and dissolution, successful oral delivery of drug from the developed dosage form is also a function of RIF and INH stability in the GI tract and interaction with concomitantly administered drugs. With the increased understanding of the factors affecting anti-TB drug dissolution and drug stability in the GI tract, it is possible to apply and interpret the dissolution methodology to understand the performance of RIF-containing formulations *in vivo*.

In addition to the established RIF degradation, Singh et al. (2000a, 2000b) reported on the *in situ* degradation of RIF and INH on exposure to acidic conditions. In this process, INH in presence of

RIF in 0.1N HCl degraded by 3.52% in 15 minutes and 10.32% in 3 hours, conferring added significance to the desire for a small intestinal mode of INH release.

Therefore, what must be demonstrated is adequate segregation of RIF and INH upon co-administration. This can be verified *in vitro* by demonstration of immediate availability and dissolution of RIF, with minimal INH release in acidic media, subsequent to simultaneous drug release testing of the combination.

Several methods are available for the individual determination of the above compounds in available capsule, tablet and suspension forms such as spectrophotometry/ colorimetry (Galal et al., 1992) chromatography (Shah et al., 1992; Mohan et al., 2003; Prabakaran et al., 2004) and microbiology (Buniva et al., 1983). The colorimetric method is widely used because of its simplicity and has been an official method for analysis of dissolution samples of FDCs containing RIF and INH and/ or PYZ (Goicoechea and Olivieri, 1999).

Chromatographic determination of RIF, INH and PYZ employing high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) has been described. Quantification of anti-TB components in pharmaceutical mixtures using normal- and reversed-phase HPLC has been delineated, with various modifications, by a number of authors (Shah et al., 1992; Mohan et al., 2003; Prabakaran et al., 2004); and its increased popularity to date arises from its ability to resolve the major degradation products of RIF, which include 3-FRSV and its interaction product with INH, rifampicin *N*-oxide, 25-desacetyl rifampicin, rifampicin quinone.

HPTLC was shown by Argekar et al. (1996) to be an effective technique for simultaneous

determination of RIF, INH and PYZ in single samples in routine analysis, employing ethyl acetate: ammonia: ethyl alcohol: cyclohexane as mobile phase on glass baked silica gel plates. Quantitative estimation was accomplished by densitometric scanning with a UV-visible detector. The limitation of this approach is borne in the requirement of sample grinding, thus single determination of drug content is successfully attained; however, drug-release evaluation, especially of modified-release dosage forms, would not be practical.

Spectrophotometric determination has gravitated towards classical least squares (CLS), and the more accurate and precise partial least squares (PLS) analysis of absorption spectra, and two-wavelength spectrophotometry. Recently RIF, INH and PYZ have been resolved employing first-derivative UV spectrophotometry using information, which is limited to a discrete number of wavelengths in order to solve a system of three equations with three unknowns (Goicoechea and Olivieri, 1999).

8.1.1. Spectrophotometric Analysis of RIF and INH Absorption Data

Multivariate calibration methods applied to absorptive and emissive spectral data are increasingly used for pharmaceutical analysis as a simple, low-cost alternative to chromatography. Absorptive data have the advantage of using full spectral information, and are useful for the resolution of complex mixtures of analytes with no need of prior separation or extraction. Full-spectrum methods usually provide significant improvement in precision over methods restricted to a small number of wavelengths. In addition, these avail the full-spectrum residuals for examination and interpretation (Goicoechea and Olivieri, 1999).

Following the resolution of mixtures of urinary metabolites of aspirin and styrene using CLS analysis of spectrofluorometric data, and of binary mixtures of antiepileptics and antihistaminics

in pharmaceutical preparations, using electronic absorption data and PLS regression, Goicoechea and Olivieri (1999) reported the successful simultaneous quantification of RIF, INH and PYZ in anti-TB FDC tablets, by applying electronic absorption measurements together with multivariate calibration analysis.

PLS regression is an extension of the multiple linear regression model, without imposing the restrictions employed by discriminant analysis, principal components regression, and canonical correlation. It is a biased, non-least squares regression procedure that relates a set of predictor variables to multiple response variables. PLS is used when predictors are highly collinear or predictors outnumber observations and ordinary least squares regression either fails or produces coefficients with high standard errors – the reason for its success in spectral analysis. In its simplest form, a linear model specifies the linear relationship between a dependent variable, Y, and a set of predictor variables. Prediction functions are represented by factors extracted from the $Y'XX'Y$ matrix. The number of such prediction functions that can be extracted typically will exceed the maximum of the number of Y and X variables.

In this application, PLS regression was used as an exploratory and analytical tool to select suitable predictor variables and to identify outliers. This approach was combined with classical linear regression for the generation of equations for RIF and INH determination in FDCs combining these drugs.

8.1.2. High Performance Liquid Chromatographic Analysis of RIF and INH

HPLC has undoubtedly grown to be the most popular and versatile of all analytical techniques in laboratories today (Ahuja, 2005). As a mode of chromatography, it is the most widely used analytical technique for quantitative determination of components of a sample.

The chromatographic process can be defined as a separation technique involving mass-transfer between stationary and mobile phases. HPLC utilises a liquid mobile phase to separate the components of a mixture. HPLC instrumentation comprises a pump, injector, column, detector and integrated computer system. The key component of the system is the column where separation of the injected analyte (in selected mobile phase) into its components transpires. Since the stationary phase is composed of micrometer size porous particles, a high-pressure pump is required to move the mobile phase through the column. Ultimately, each component elutes from the column as a narrow band (or peak) digitally captured by intricate computer software. Detection of the eluting components may be selective or universal, dependent on the detector employed. The amount of resolution is important, and is dependent upon the extent of interaction between the solute components and the stationary phase in the column. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases.

As a result, HPLC possesses a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures. A number of HPLC configurational approaches to the simultaneous determination of RIF and INH have been described and are summarised in Table 8.1.

Prabakaran et al. (2004) developed a reverse phase HPLC method for simultaneous estimation of RIF and INH in dissolution fluids. Two well-separated peaks were obtained for RIF (retention time, 1.75 minutes) and INH (retention time, 2.3 minutes).

The recently notified USP gradient HPLC method for quantitative determination of RIF, INH and PYZ in FDC formulations has been tested and evaluated by various researchers to determine its

ability to resolve major degradation products of RIF, which include 3-FRSV and its isonicotinyl hydrazone interaction product, rifampicin *N*-oxide, 25-desacetyl rifampicin, and rifampicin quinone (Mohan et al., 2003).

Table 8.1: Specifications for HPLC methodologies

Specifications	USP 24 (RIF and INH Capsules) Method	USP Gradient Method^a	Reverse Phase Method
<i>Column</i>	L1 detector and a 4.0mmx30cm column	L1 column (250x4.6mm)	C-18 reverse phase column (250x4mm) was used
<i>Particle size</i>	10µm	5µm	5µm
<i>Injection volume</i>	50µL	20µL	20µL
<i>Mobile phase</i>	Filtered and degassed mixture of water, Phosphate buffer solution and methanol (850:100:50).	-Buffer solution: 1.4 g dibasic sodium phosphate in 1L water (pH 6.8) and acetonitrile (ACN) -Solution A = Buffer: ACN = 96:4 -Solution B = Buffer: ACN = 55:45 ^a	Methanol and 0.01 M potassium dihydrogen phosphate (82.5:17.5)
<i>Flow rate</i>	1.5mL/min	1.0mL/min	1.0mL/min
<i>Detection wavelength</i>	254 nm (for INH)	238nm (for both drugs)	254 nm (for both drugs)

^a See Table 8.2 for gradient program

The parameters, with some modifications suggested by Mohan et al. (2003), specified in the USP in a monograph covered recently in a current interim revision announcement for gradient HPLC analysis of three anti-TB drugs in a four-drug combination product, is shown in Table 8.2.

Table 8.2: Gradient program prescribed in the USP method (Anonymous, 2001)

Time (min)	Solution A (%)	Solution B (%)	Elution
0	100	0	Equilibration
0-5	100	0	Isocratic
5-6	100→0	0→100	Linear gradient
6-15	0	100	Isocratic

Mohan et al. (2003) found it of interest to evaluate whether the USP method for combined assay of RIF, INH and PYZ was able to simultaneously resolve the three drugs and known degradation products of RIF including 3-FRSV. Their findings revealed that the method could separate the three drugs from most known and major degradation products of RIF only if stringent specifications were set, as the nature of separation varied with the make of the columns.

8.2. Materials and Methods

8.2.1. Materials - Commercially Available FDCs

The optimum multiparticulate formulation, comprising enterospheres equivalent to 75mg INH and reconstitutable granules equivalent to 150mg RIF (dispersed in 25mL double-deionised water immediately prior to testing), was evaluated for comparison with commercially available FDCs. Drug content of the reconstitutable granules was spectrophotometrically validated in 0.1M HCl at 235nm. FDC formulations utilised in this study were film-coated four-drug FDC tablets consisting of 150mg RIF, 75mg INH, 400mg PYZ and 275mg ETB and two-drug FDCs containing 300mg RIF and 150mg INH, and 150mg RIF and 100mg INH, purchased from Aventis[®] (Aventis Pharma (Pty) Ltd. Midrand, Gauteng, South Africa). All formulations employed were according to WHO recommended dose ratios and the strength was according to four-drug FDC tablets incorporated in WHO list of essential medicines (Blomberg et al., 2001). Only two-drug FDCs were instituted in regressional analyses applied to spectrophotometric absorbance data. RIF, to serve as a reference standard, was obtained from Themis Chemicals[®] (Goregaon, Wadeville, South Africa). INH (reference standard) was purchased from Aldrich[®] (Sigma-Aldrich Inc., St. Louis, USA).

8.2.2. *In Vitro* Drug Release Testing

Dissolution testing was in accordance with the USP 24 drug release testing specification for RIF and INH Capsules (USP 24, 2000). *In vitro* drug release from the formulations was studied employing USP apparatus II (rotating speed: 50 rpm) in a six-station dissolution apparatus (Caleva[®], Model 7ST) in 1000mL 0.1M HCl (pH 1.2 at 37±0.5°C) as dissolution medium. Balancing withdrawal of 1mL (for UV analysis) or 5mL (for HPLC analysis) filtered samples (0.45µm filter, Millipore, Billerica, USA) was undertaken at 15, 30, and 45 and 120-minute time intervals, suitably diluted, and analysed immediately by UV spectrophotometry, or HPLC with colorimetric determination of RIF. Immediate analysis (within at least 3 hours) was necessary due to the instability of RIF in acidic media. For each dissolution test, the sixth vessel was used as a reference vessel in which pure drugs equivalent to the amount present in the formulation were dissolved.

8.2.3. Quantitative Analytical Determination of RIF and INH by Regression Analysis of Spectrophotometric Absorption Data

Electronic absorption measurements were carried out on a Specord 40 spectrophotometer (Analytik Jena AG, Jena, Germany), using 1.00cm quartz cells. All spectra were saved in ASCII format, and transferred for regression analysis. PLS and classical regression analysis were performed by importing the spectral files to a statistical programme and processing them with the PLS-1 analysis, regression and fitted line plot/ standard curve fit function. Spectral data in the UV-range of 190-300nm were subjected to PLS-1 analysis, selecting an optimum spectral region for each component. RIF possessed a peak absorbance at 235nm in 0.1M HCl and INH, at 265nm.

The statistical programme (MINITAB[®], V14, Minitab, USA) employed in the analyses applies

the nonlinear iterative partial least squares (NIPALS) algorithm. The algorithm reduces the number of predictors using a technique similar to principal components analysis to extract a set of components that describes maximum correlation among the predictors and response variables. It then performs least squares regression on these uncorrelated components.

In order to obtain the calibration matrix for applying PLS and classical regression analysis, solutions of each of the pure components and the RIF-INH mixture in 0.1M HCl (pH 1.2) were prepared, with the concentrations lying in the linear range (maximum concentrations: 30mg/L for RIF and 15mg/L for INH). The absorbance data (in the range 190–300nm, digitised every 1.0nm, 111 points per spectrum) were stored for subsequent application of regression methodology. The following approach was adopted for application to absorbance data:

1. PLS analysis of the matrix of absorbance data was applied to RIF and INH components and the RIF-INH mixture or test samples to facilitate model selection, and for calibration matrix generation
2. The components that adequately predicted the amounts of RIF and/ or INH were identified from the square of the correlation coefficient (R^2), which furnished an indication of the quality of fit of all the data. Drug content determination in FDCs was undertaken to verify method validity and ascertain whether RIF and INH amounts in FDC tablets were within USP 22 specifications.
3. Classical regression analysis for identification of the line of best fit in the optimal spectral region was undertaken for the generation of equations for RIF and INH determination at each sample point.

8.2.4. Quantitative Analytical Determination of RIF and INH by Colorimetry and HPLC

Each HPLC analytical approach delineated in Table 8.1 was investigated for its ability to achieve

adequate identification of the RIF and INH components. Poor separation of the INH peak from the solvent was attained following institution of the USP Gradient Method and Reverse Phase Method, acquiescing inadequate INH detection, with particular reference to the developed modified-release optimum multiparticulate formulation. The USP method for analysis of RIF and INH Capsules (USP 24, 2000) was thus employed.

The PBS was prepared following dissolution of 15.3g of dibasic potassium phosphate and 80.0g of monobasic potassium phosphate to 1L in double-deionised water. An INH standard solution was prepared by accurately weighing 66mg INH (reference standard) into a 100mL volumetric flask, dissolving in, and diluting with, 0.1M HCl to volume. A standard stock solution was prepared following the accurate weighing of 66mg RIF (reference standard) into a 200mL volumetric flask and dissolving in 10mL of 0.1M HCl. 50mL of the INH standard solution was added, diluted with 0.1M HCl to volume, and mixed.

At the end of the dissolution test run, a 5mL aliquot of the standard stock solution and 10mL of phosphate buffer solution was transferred to a 50mL volumetric flask and diluted with double deionised water to volume. Simultaneously, a 10mL aliquot of the test solution was withdrawn and filtered. This was allowed to cool for 10 minutes. 5mL of the filtrate and 10mL of the phosphate buffer solution were transferred to a 50mL volumetric flask and diluted with deionised water to volume. These solutions were analysed immediately, if possible, otherwise within 3 hours of final dilution. The amount of RIF dissolved was ascertained colorimetrically from the absorbance at the wavelength of maximum absorption in the visible region (475nm) for the standard and test solution. The chromatographic system for INH detection was as per Table 8.1 for RIF and INH capsules. Equal volumes ($\approx 50\mu\text{L}$) of the standard solution and the test solution were separately injected into the chromatogram. The response for the INH peak was measured.

8.3. Results and Discussion

8.3.1. Spectrophotometric Analysis

Confirmation of RIF degradation was by overnight (12 hour) incubation of a RIF-INH mixture at simulated gastric pH. There was a significant reduction in peak absorbances in the range 190-300nm of the composite solution (up to 65.26%) suggestive of degradation (Figure 8.1). The presence of the isonicotinyl hydrazone of 3-FRSV degradation product was confirmed by UV spectrophotometric detection and quantification in an extraction with *n*-hexane: ethyl acetate (2:1) (Florey, 1976). For the optimum formulation, degradation, as observed by reductions in peak absorbances did not exceed 15.32% after combined incubation in acidic media for 12 hours. Isolation of the INH from the RIF in acidic media was thus effectively achieved by entrapment in enterospheres, even for extended periods.

From the electronic absorption spectra of the RIF-INH mixture (Figure 8.1(c)), a degree of spectral overlap is observed in the useful region of 200–300nm. The method instituted for resolving these mixtures was PLS regression. PLS regression and classical regression analysis was performed for each of the RIF-INH combinations. From the graphs generated (Figures 8.2 to 8.4), model selection was facilitated and the appropriate regression equations were implemented for drug release prediction.

The predicted drug content for the FDC tablets was acceptable within the USP 22 specifications confirming method validity (Table 8.3). Examination of the regression coefficients of the resultant models generated by PLS demonstrated adequate prediction of the RIF component in FDCs due to the ability to reproducibly generate a calibration matrix in the region of interest ($R^2 \geq 85.9\%$), except for Rifanah-150 where the INH component was satisfactorily predicted with improved component differentiation.

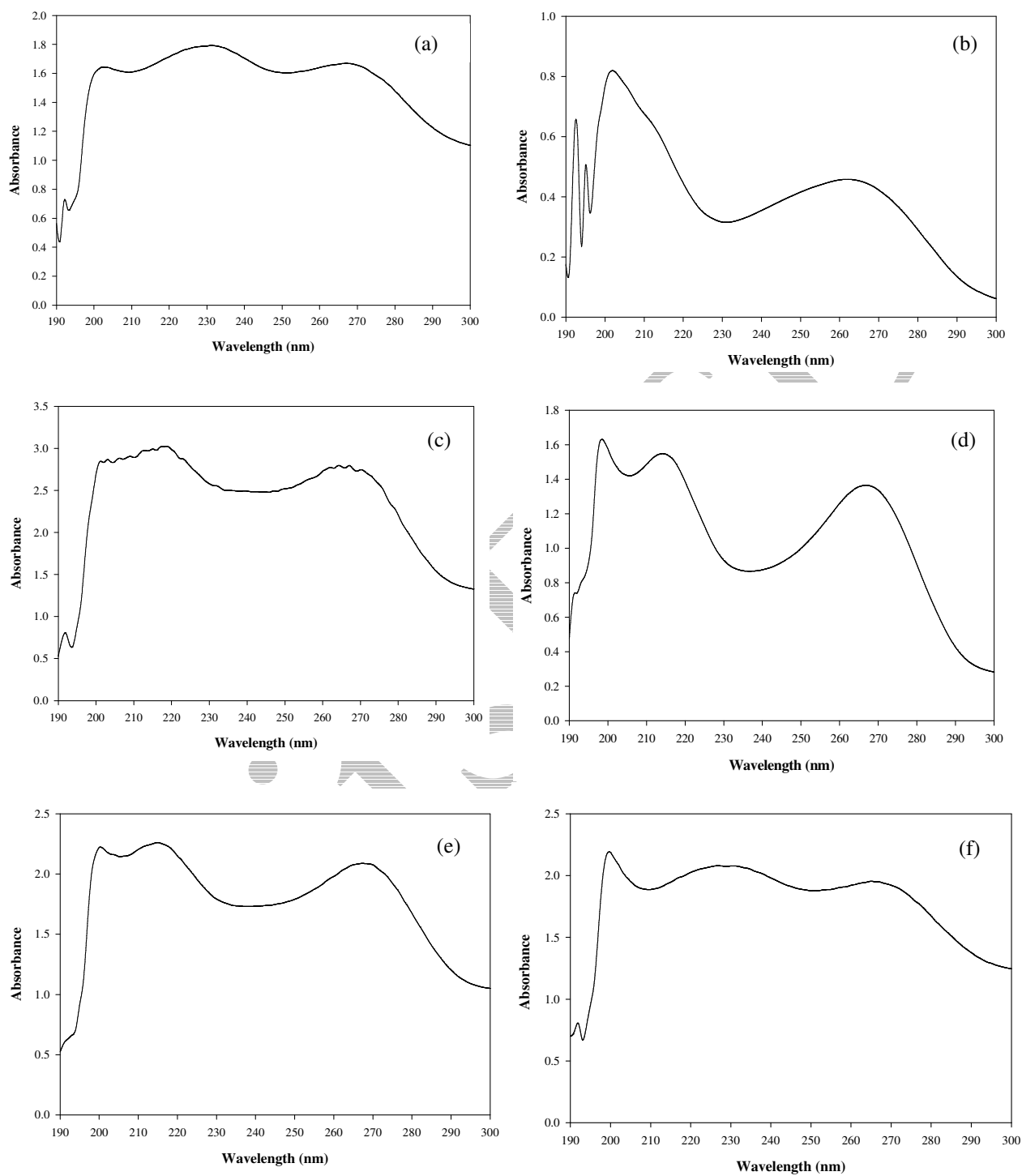


Figure 8.1: UV spectra: (a) RIF, (b) INH, (c) RIF-INH at t_{0h} and (d) t_{12h} representing extent of RIF degradation in acidic media, (e) optimum formulation at t_{0h} and (f) t_{12h} in 0.1M HCl

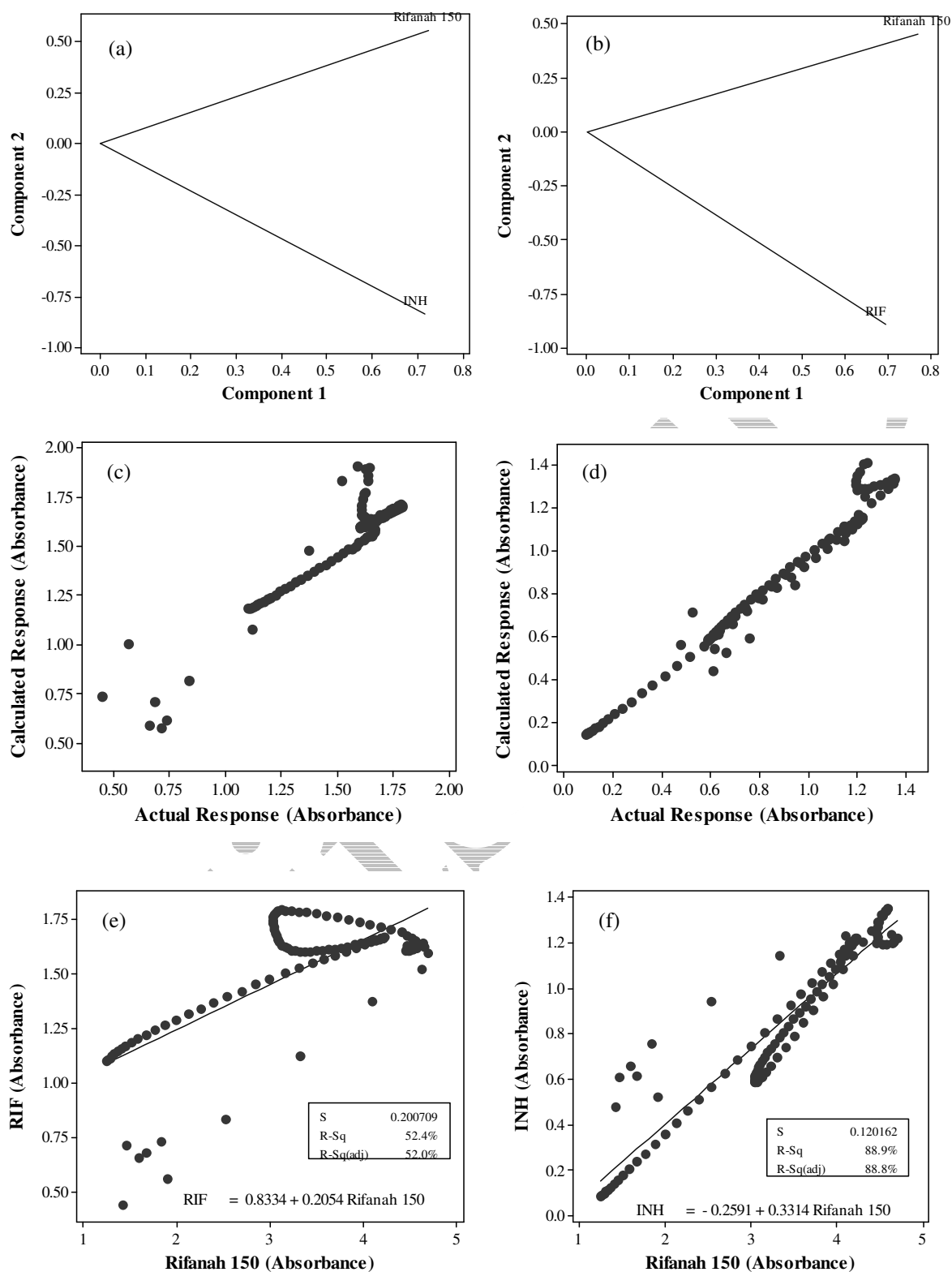


Figure 8.2: PLS analysis of spectrophotometric absorbance data for Rifanah 150: PLS loading plot for (a) RIF and (b) INH; response plot for response (c) RIF (2 components) and (d) INH (2 components); fitted line plot for (e) RIF and (f) INH

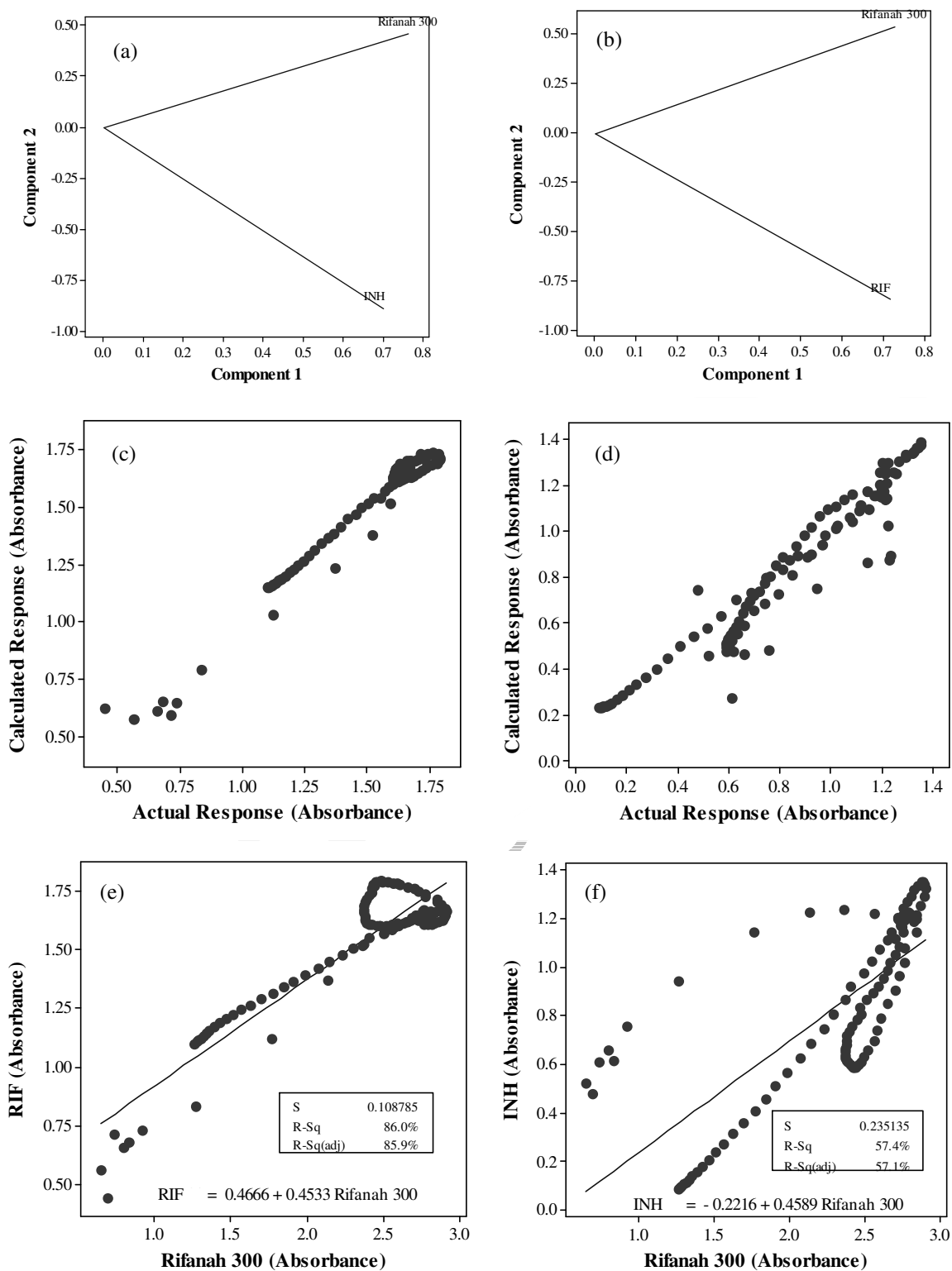


Figure 8.3: PLS analysis of spectrophotometric absorbance data for Rifanah 300: PLS loading plot for (a) RIF and (b) INH; response plot for response (c) RIF (2 components) and (d) INH (2 components); fitted line plot for (e) RIF and (f) INH

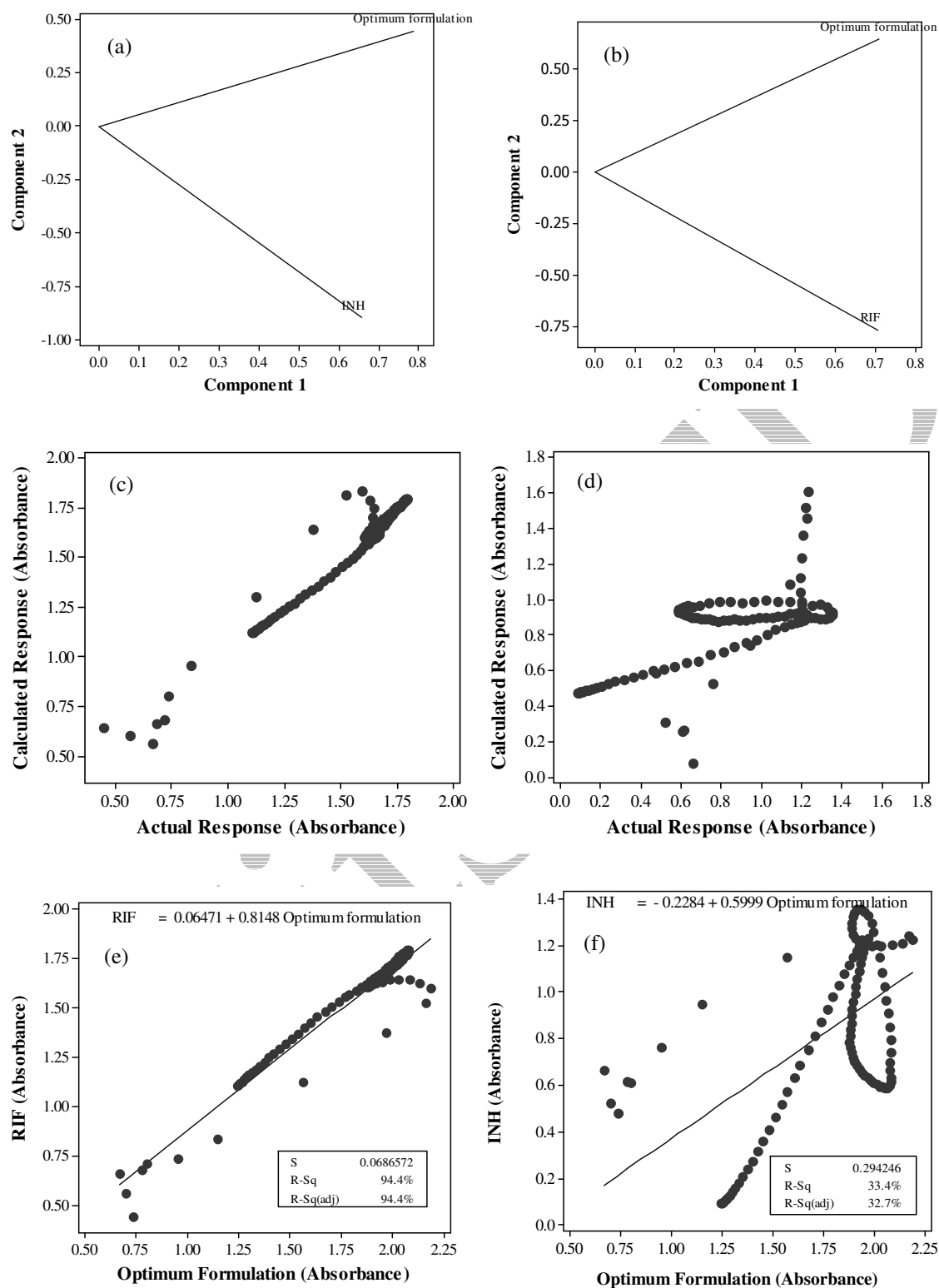


Figure 8.4: PLS analysis of spectrophotometric absorbance data for optimum RIF-INH dispersible multiparticulate system: PLS loading plot for (a) RIF and (b) INH; response plot for response (c) RIF (2 components) and (d) INH (2 components); fitted line plot for (e) RIF and (f) INH

Table 8.3: Drug content validation in FDC tablet formulations

	Rifanah-150		Rifanah-300	
	RIF	INH	RIF	INH
Actual average content of each component in tablet (mg)	148.93	78.44	298.27	144.50
Amount reported by manufacturing laboratory (mg)	150.00	75.00	300.00	150.00
% of the reported content	99.29	104.59	99.42	96.33
% of the range accepted by the USP 22 (1990)	90-130	90-110	90-130	90-110

Table 8.4: Model selection and validation and regression coefficients for RIF release prediction

Sample	Component	R ²	Regression Coefficient (standardised)
Rifanah-150	INH	0.4052	-1.787
	RIF-INH	0.8789	2.408
Rifanah-300	INH	0.6748	-0.515
	RIF-INH	0.9729	1.318
Optimum Formulation	INH	0.8067	-0.116
	RIF-INH	0.9533	1.039

Table 8.5: Model selection and validation and regression coefficients for INH release prediction

Sample	Component	R ²	Regression Coefficient (standardised)
Rifanah-150	RIF	0.7071	-0.417
	RIF-INH	0.9717	1.245
Rifanah-300	RIF	0.4391	-1.566
	RIF-INH	0.9175	2.211
Optimum Formulation	RIF	0.2901	-1.388
	RIF-INH	0.4409	1.927

Regression equations generated employing the statistical coefficients (Table 8.4) satisfactorily predicted RIF release at each sampling point ($R^2 \geq 95.3\%$). The ability of PLS models to predict the INH component was less satisfactory (Table 8.5), owing to extensive overlap in the peak spectral region of INH. Indirect determination of INH from regression models following RIF prediction yielded reproducible results.

The novel developed optimum formulation comprising reconstitutable and enterosoluble multiparticulate entities demonstrated adequate segregation of RIF and INH delivery as compared to commercially available two-drug FDCs (Figure 8.5). Administration of the INH-loaded enterospheres as a dispersible suspension system instigated a slight lag in the INH release from the enterosphere noted in the first 15 minutes of media exposure, with the co-administered suspension contributing bulk to the simulated gastric contents, somewhat hindering enterosphere matrix hydration and drug diffusion. The suspension system ensured sufficient availability of RIF in solution, excluding notable binding of RIF by gel-forming constituents. Both RIF and INH were rapidly and completely released from Rifanah-150 within 30 minutes. After 45 minutes, the total drug content of Rifanah-300 was available. Neither formulation could be expected to control INH bioavailability in the gastric environment due to their immediate-release nature.

Demonstration of adequate RIF release from the two-drug FDCs in acidic media was essential. Because RIF is less soluble in the proximal intestinal contents, rapid release in the acidic environment of the stomach, in which the borderline class II drug demonstrates favourable solubility, also contributes crucially to RIF's ultimate bioavailability (Agrawal and Panchagnula, 2004).

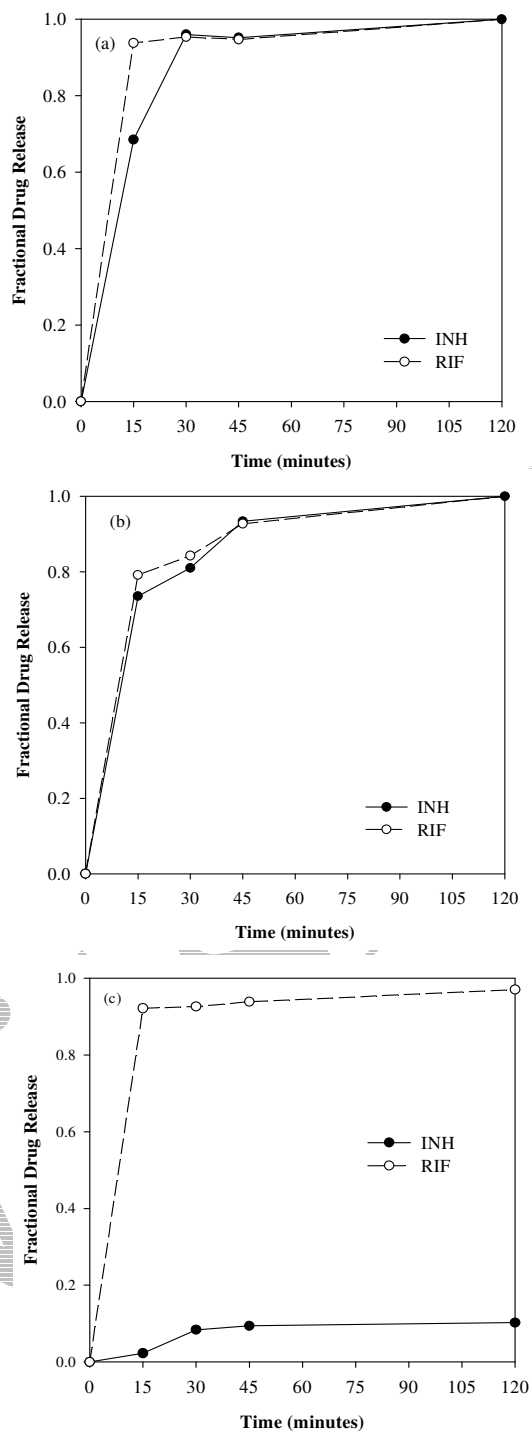


Figure 8.5: Drug release from FDCs following regression analysis of UV spectrophotometric absorbance data: (a) Rifanah-150, (b) Rifanah-300, and (c) the optimum RIF-INH multiparticulate system (S.D.s were within ± 0.127 for RIF release and within ± 0.155 for INH release in all cases)

8.3.2. Colorimetric and High Performance Liquid Chromatographic Analysis

Systems prescribed for simultaneous chromatographic determination of RIF and INH in pharmaceutical FDC combinations demonstrated poor separation of the INH component from the solvent, even with specified modifications. This is a common observation, as separation and system suitability requirements of the official method are not always met during actual studies using different columns and instruments. Mohan et al. (2003) commented on these reported impediments and referred to personal communications where users had encountered such difficulties. Modifications to prescribed methods have been reported, however, even these were not successfully implemented with available columns and instrumentation. Purported reasons for this being that:

1. The nature of separation varied with the make of the columns - column specifications needed to be fixed more exactly, especially where degradation products are formed
2. The advised minimum theoretical plates for RIF were met, however, this was not the case with INH
3. With particular relevance to this investigation, prescribed HPLC methods may not be suitable for detection of the low concentrations of INH released into the dissolution media from the modified-release form i.e. the INH-loaded enterospheres

Chromatographic determination of INH and colorimetric determination of RIF at 475nm was adequately implemented on dissolution samples of the two-drug FDCs. However, a strong possibility existed for overestimation of RIF if there was formation of the isonicotinyl hydrazone of 3-FRSV in the sample and direct analysis was done colorimetrically at 475nm. Mariappan et al. (2004) showed that the isonicotinyl hydrazone of 3-FRSV possessed a similar colorimetric spectrum to that of RIF and indicated that this hydrazone possessed one-third the absorptivity of RIF. The analysis of combinations of RIF and the hydrazone revealed that RIF could be

overestimated to a maximum extent of 33%, while interference varied at other relative ratios of the two compounds. Thus immediate analysis of dissolution samples was necessitated with the final sampling point at 60 minutes, after which the accuracy and reliability of RIF readings was unsatisfactory. This method would thus prove ineffective for analysis of stability samples.

A minor modification was applied to the dissolution testing conditions specified in the USP 24 method based on the acknowledgement that the appropriate rotational speed of the paddle employed in testing was of considerable importance in assuring that the procedure was useful and discriminatory. It has been elucidated that alteration of the agitation speed affected the ability of an *in vitro* test to distinguish *in vivo* performance for both immediate- and more extended-release formulations. The agitation intensity employed for the paddle apparatus was reduced from 100rpm prescribed in the pharmacopoeial standard for RIF and INH capsules (USP 24, 2000) to 50rpm. Agrawal and Panchagnula (2004) have found an agitation intensity of 50rpm to be optimal for the discrimination of release of RIF from FDC formulations, and dissolution profiles obtained at this prescribed rotational speed were analytically interpreted.

There was an acceptable degree of correlation between the drug release data, as predicted by regression analysis of UV spectrophotometric data, and chromatographic and colorimetric determination of INH and RIF respectively. The agreement between the RIF release data as obtained by the afore-described methods for the two-drug FDC and the optimum multiparticulate formulation was good ($R^2 = 0.9976$ and $R^2 = 0.9996$). The degree of agreement between the INH release data was lower ($R^2 = 0.9793$ and $R^2 = 0.9739$), but satisfactory, nonetheless.

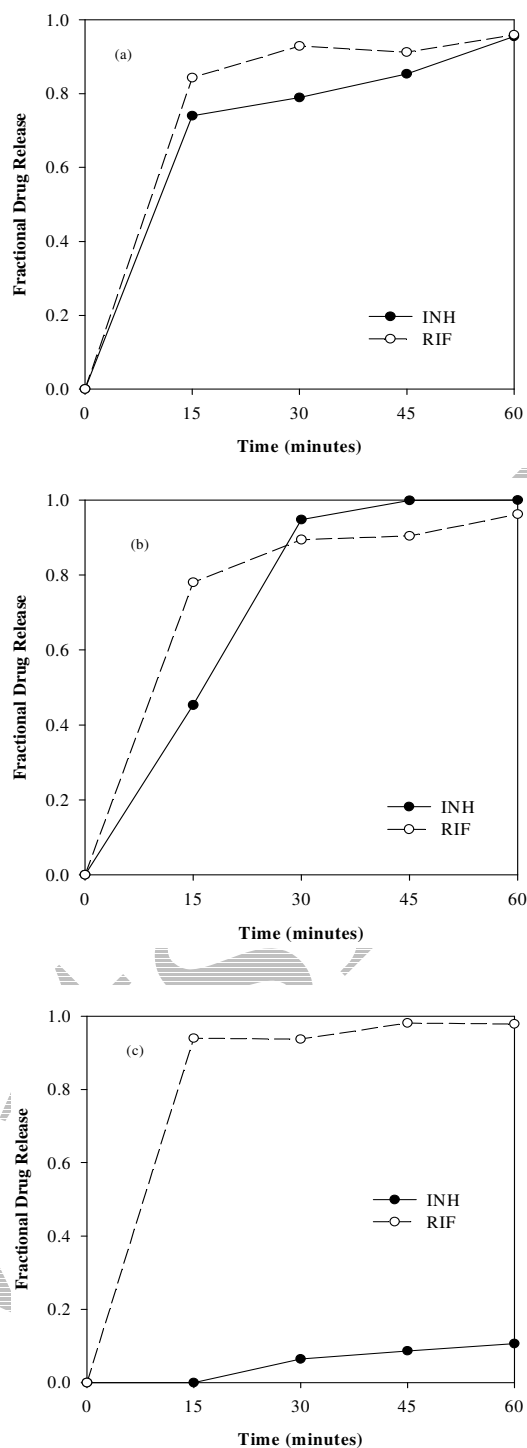


Figure 8.6: Drug release from FDCs following chromatographic determination of INH and colorimetric determination of RIF: (a) Rifanah-150, (b) Rifafour-275, and (c) the optimum RIF-INH multiparticulate system, where the area of the standard peak in the INH chromatogram was 126.9 (S.D.s were within ± 0.134 for RIF release and within ± 0.071 for INH release)

8.4. Concluding Remarks

Regression analysis of UV spectrophotometric data effectively validated the drug content of RIF-INH FDCs, within USP 22 specifications. PLS regression and classical regression analysis revealed that the RIF component of the FDC could be effectively predicted from generation of a calibration matrix and UV spectral readings of the RIF-INH combination at each sampling point were satisfactory for calculating RIF release from the dosage form. Fractional release of RIF was thus calculated directly employing the generated regression equations. Prediction of INH release was less effective, especially from the modified-release multiparticulate formulation, essentially due to the high degree of spectral overlap of RIF with INH, which complicated INH detection at low concentrations. Indirect determination of INH after RIF computation was thus most accurate. These investigations established adequate segregation of RIF and INH release from the novel optimum multiparticulate formulation in the simulated gastric environment.

Chromatographic and colorimetric determination of INH and RIF respectively, as prescribed in the USP standard for RIF and INH capsules (USP 24, 2000) provided a satisfactory means for the analysis of dissolution samples of two-drug FDCs. However, the possibility of overestimation of the RIF in the presence of the hydrazone degradation product when determined colorimetrically at 475nm must be borne in mind, with particular reference to the period of time lapsing between dissolution sample withdrawal and analysis.

Because commendable correlatory behaviour was observed between the two investigated methods for RIF and INH determination in two-drug FDCs, regression analysis of UV spectrophotometric data for simultaneous RIF and INH prediction thus provides a simplified methodology for use in resource-poor countries for the assurance of RIF bioavailability from FDC formulations intended for directly observed treatment-shortcourse (DOTS).