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RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF ISONIAZID AND PARAAMINO SALICYLIC ACID SIMULTANEOUSLY IN PREPARED COCRYSTAL FORMULATION BY UV SPECTROPHOTOMETRY

ABSTRACT

Isoniazid (INH) and Paraamino salicylic acid (PAS) are antitubercular drugs. Three simple, sensitive, precise and economical simultaneous UV spectrophotometric methods have been developed and validated in bulk drugs and its co-crystal formulation. Co-crystals of INH & PAS were prepared by solvent evaporation technique. Methanol was selected as the solvent. Maximum absorption was observed at 263nm and 271nm for INH and PAS respectively. The Method A was based on simultaneous estimation by simultaneous equation method. Method B was based on calculation of Area under Curve (AUC) for the analysis of INH and PAS in the wavelength range of 258-268nm. In Method C, First order derivative spectra of INH and PAS showed a sharp peak at 243 and 257 nm respectively. All the three methods were validated as per ICH guidelines. The proposed methods were applied to estimate the amount of INH and PAS in bulk drug and formulations.

Keywords Isoniazid, Paraamino salicylic acid, simultaneous equation method, Derivative spectroscopy, Area under Curve (AUC).

1. INTRODUCTION

Isoniazid (INH) (Figure 1.a) is a first line antitubercular drug, activated by bacterial catalase. Paraamino salicylic acid (PAS) (Figure 1.b) is a second line antitubercular drug inhibiting the folic acid synthesis (1). Clinical trials suggest combination of INH & PAS do have a synergistic effect. Co-crystals are defined as multiple component structures whose components interact by non-covalent interactions such as hydrogen bonding or other weak intermolecular interactions rather than by ion pairing (2,3). Co-crystals enhance pharmaceutical properties by modification of solubility, dissolution rate, chemical stability, mechanical behavior, moisture uptake and bioavailability (4). Co-crystals of PAS & INH was prepared and formulated into a tablet dosage form. (5-7)

Literature survey revealed that developed and validated rapid, sensitive and specific methods for the determination of INH and PAS in individual dosage forms have been reported (8). Simultaneous estimation methods have not been so far reported for the co-crystals of INH and PAS. The present research work describes preparation of cocrystals of INH, PAS and three simple, precise, accurate and economical simultaneous UV spectrophotometric methods for the estimation of INH and PAS in bulk and co-crystal dosage form.

2. MATERIALS AND METHODS

INH was purchased from Loba chem. Pvt Ltd, Mumbai. PAS from Hi media pvt.Ltd, Mumbai, methanol was purchased from Merck pvt. Ltd, Mumbai. Shimadzu double beam UV- visible spectrophotometer UV- 1700, Japan was used for the study. In addition an electronic analytical balance (Shimadzu, Japan) and scanning electron microscope (Zeiss electron microscope, EVO MA 15) was used.

2.1 Preparation of crystal form INH-PAS (1:1) co-crystal by solvent evaporation method:

Isoniazid (0.137g, 1 milli mol) and Paraamino salicylic acid (co-crystal former, 0.153g, 1 milli mol) were dissolved separately in 5 ml of methanol with warming and mixed together. Solution was cooled to room temperature and kept for slow evaporation for six hours. The crystals were isolated through a membrane (0.45µm) and dried in the air.

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2.2 Development of Method

2.2.1 Selection of solvent and wavelength

For all the three methods methanol was selected as the solvent. From the spectra, two wavelengths were selected as 263, 271 nm for INH and PAS respectively (Figures 2.a, 2.b).

2.2.2 Method A: Simultaneous equation method

Serial dilutions of solutions ranging from 2-10 µg/ml were prepared and scanned under UV (Table 1). The standard stock solutions of concentration of 10µg/ml of INH and PAS and the formulation were prepared on diluting with methanol. These solutions were scanned over the range of 200-400nm (Table 2). The concentration of INH and PAS was calculated by following equations, (9)

$$C_{INH} = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$C_{PAS} = \frac{A_2\, a_{x1} - A_1\, a_{x2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

2.2.3 Method B: Area under Curve Method

From the spectra of two drugs, area under the curve in the range of 268- 258nm and 276-266nm were selected for the analysis (Figures 3.a, 3.b). Series of concentrations ranging from 2-10 µg/ml solutions were prepared and record the spectras and the calibration curves were prepared at AUC range (10). By applying Cramer's rule, the concentration of the sample solution can be determined (Table 3).

2.2.3 Method C: First Derivative Spectroscopy

In this method, 10µg/ml solution of both Isoniazid and Paraamino salicylic acid were prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. The absorption spectra thus obtained were derivatized from zero to first order. First order derivative spectra were selected for analysis of the drug (11). First order derivative spectra of two drugs showed a sharp peak at 243 and 257 nm respectively as shown in figures which were selected for its quantification. The first order derivative spectras were taken for the solutions ranging from 2-10µg/ml of INH and PAS (Figures 4.a, 4.b).

2.3 Validation

The method of analysis was validated as per the recommendations of ICH (12) and USP (13) for the parameters like accuracy, linearity, precision, detection limit and quantitation limit. The accuracy of the method was determined by analysis of formulation. Intraday and interday precision study of INH and PAS was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of 2 µg/ml and 6 µg/ml and 10 µg/ml of INH and PAS, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve. (Tables 4, 5).

3. RESULTS AND DISCUSSION

Co-crystals of INH and PAS were prepared and characterized. INH and PAS exhibited maximum absorption at 263nm and 271nm respectively and obeyed Beer's law in the concentration range of 2-10µg/ml. The accuracy of the methods was assessed by recovery

studies at 50% and 100% levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives.

4. CONCLUSION

It can be concluded that simultaneous equation method, AUC Method and First Derivative spectroscopy Methods were developed for estimation of prepared and characterized co-crystals. All the three methods provide a convenient and accurate way for analysis of co-crystals of INH and PAS. The developed methods were found to be simple, sensitive, accurate, precise, and reproducible and can be used for simultaneous estimation of Isoniazid and Paraamino salicylic acid in bulk drugs and co-crystal formulations.

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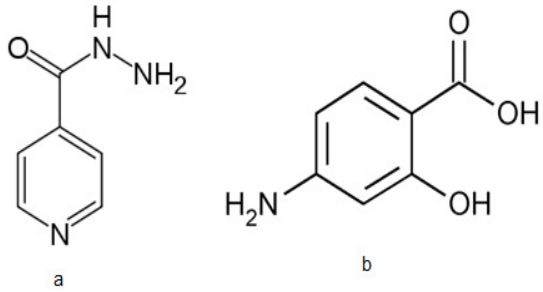

Fig 1: Structure of a) Isoniazid and b) Para amino salicylic acid

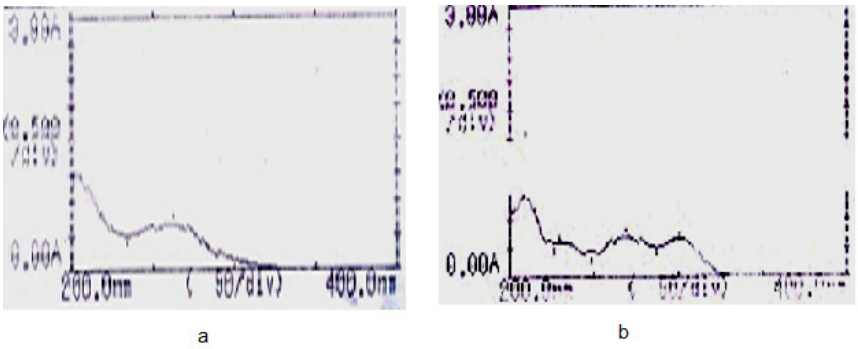

Fig 2: UV spectrum of a) Isoniazid and b) Para amino salicylic acid in methanol

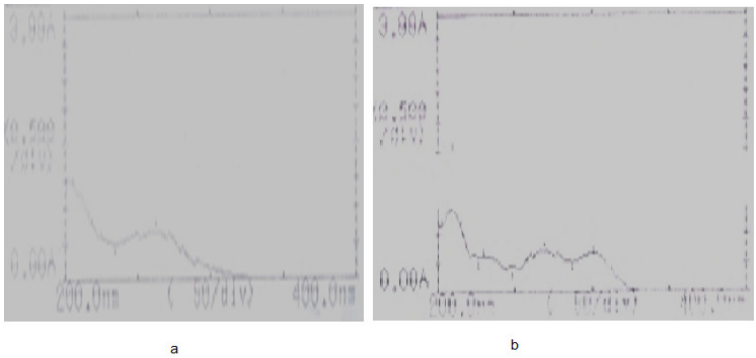

Fig 3: AUC graph for a) Isoniazid and b) Para amino salicylic acid

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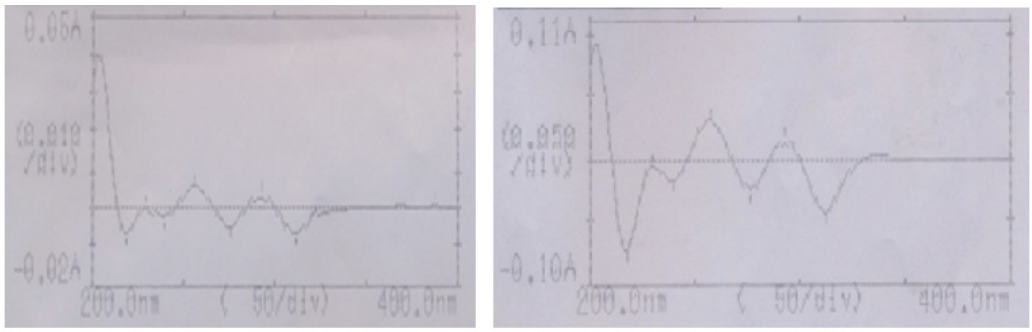

a b

Fig 4: 1st Order derivative spectra for a) Isoniazid and b) Para amino salicylic acid

Conc. (µg/ml)	INH				PAS			
	Absorbances		Absorptivities		Absorbances		Absorptivities	
	263nm	271nm	263nm	271nm	263nm	271nm	263nm	271nm
2	0.1932	0.2010	0.0965	0.1005	0.1130	0.1390	0.0565	0.0695
4	0.2564	0.2693	0.0640	0.0673	0.2223	0.2465	0.0555	0.0616
6	0.3895	0.4056	0.0646	0.0676	0.3152	0.4321	0.0525	0.0720
8	0.4563	0.4926	0.0579	0.0615	0.4495	0.5493	0.0561	0.0686
10	0.5966	0.6088	0.0596	0.0608	0.5196	0.6258	0.0519	0.0625

Table 1: Absorbances and Absorptivities of INH and PAS at Selected Wavelengths

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Drug	**Absorbances**		**Absorptivities**	
	λ1=263nm	λ2=271nm	λ1=263nm	λ2=271nm
INH(10µg/ml)	3.2432	4.826	0.3243 **(ax_1)**	0.4826 **(ax_2)**
PAS(10µg/ml)	6.830	8.425	0.6830 **(ay_1)**	0.8425 **(ay_2)**
FORMULATION (10µg/ml)	0.79 **(A_1)**	0.897 **(A_2)**		

Table 2: Analysis of Formulation

Conc. (µg/ml)	**INH**				**PAS**			
	AUC		**Absorptivites**		**AUC**		**Absorptivities**	
	(268-258nm)	(276-266nm)	(268-258nm)	(276-266nm)	(268-258nm)	(276-266nm)	(268-258nm)	(276-266nm)
2	1.4305	1.3488	0.7152	0.6724	2.9688	2.9526	1.4844	1.4763
4	2.2126	1.9323	0.5531	0.4830	4.8789	4.8199	1.1219	1.2049
6	3.3311	2.9351	0.5551	0.4891	6.8893	6.9103	1.1482	1.1517
8	4.4274	3.9053	0.5534	0.4881	9.0994	9.3836	1.3638	1.1729
10	5.5394	4.9032	0.553	0.4903	10.685	11.803	1.0685	1.1803
Slope	0.555	0.490			1.109	1.180		
Mean			0.5861 **(ax_1)**	0.5245 **(ax_2)**			1.1919 **(ay_1)**	1.0372 **(ay_2)**
*SD			0.00719	0.00826			0.0166	0.0188
*% RSD			1.2203	1.5709			1.3922	1.8312
*R^2 value	0.998	0.999			0.995	0.996		

Table 3: Regression Analysis of Calibration Curves and Absorptivity Values of INH And PAS

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PARAMETERS	**INH**		**PAS**	
λ_{max}	263 nm	271nm	263 nm	271nm
Beer's law limit	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml	2-10 µg/ml
Regression equation (Y = mx + c)	y =0.060x+ 0.01195	y = 0.061x+ 0.00838	y =0.05300x + 0.00614	y = 0.06307x + 0.00643
Slope (m)	0.060	0.061	0.05300	0.06307
Intercept (c)	0.01195	0.00838	0.00614	0.00643
Correlation coefficient (r)	0.998	0.998	0.997	0.997
Interday precision (%RSD)	1.5326	1.4356	1.0226	0.6536
Intraday precision	0.1742	1.1524	1.7598	0.3899
LOD Value	0.042	0.065	0.225	0.131
LOQ Value	0.133	0.2	0.703	0.409

Table 4: Method Validation Parameters of INH and PAS

Level	**% Recovery**		**% RSD**	
	Formulation		**Formulation**	
	263nm	**271nm**	**263nm**	**271nm**
50%	91.08	91.31	0.5342	0.6439
100%	94.72	97.42	0.4638	0.7630

Table 5: Recovery Studies

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