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Development and Validation of Spectroscopic Method for Simultaneous Estimation of Allopurinol and α-Lipoic Acid in Combination Tablet.

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Research Article

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ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Allopurinol and α-Lipoic Acid in combination tablet dosage form using AUC & Absorbance correction method. In Area Under Curve and Absorption correction method,α-Lipoic Acid has less Absorbance compare to Allopurinol for 10 to 50 ppm so in this method we consider area under curve at 310 nm to 390 nm in case of α-Lipoic Acid which found linear in given range of concentration and there is no interference of Allopurinol in this region because it has no absorbance in this region. Allopurinol is estimated by absorption correction method at 250 nm. Methanol used as solvent. Linearity was observed in the concentration range of 10-50 µg/ml for both Allopurinol and α-Lipoic Acid with correlation coefficient 0.9998 and 0.9999 for Allopurinol and α-Lipoic Acid respectively. Mean recovery were found to be 101.35% and 101.41% for Allopurinol and α-Lipoic Acid, respectively. The proposed method was successfully applied for the simultaneous estimation of both drugs in combination tablet.

INTRODUCTION

Allopurinol and α- Lipoic acid combination used in treatment of gout and Hyperuricemia. Allopurino lis a purine analog; it is a structural isomer of hypoxanthine (a naturally occurring purine in the body) and is an inhibitor of the enzyme xanthine oxidase. Xanthine oxidase is responsible for the successive oxidation of hypoxanthine and xanthine, resulting in the production of uric acid [1,2]. It ischemically tautomeric mixture of 1*H*-pyrazolo[3,4-*d*] pyrimidin-4-ol and 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one. Allopurinol is a white or almost white, crystalline powderhaving molecular weight 136.11 g/mol [3,4]. Structure of Allopurinol Shows in Fig No.1. α- Lipoic Acid also known as Lipoic acid and Thioctic acid. α-Lipoic Acid is an Anti Oxidant. It neutralizes free radical Prevent damage to the cell which release more uric acid. It also makes sensefor that our bodies to produce less of our own antioxidant, uric acid, when we give antioxidant externally [5,6,7]. It is chemically 1,2-Dithiolane-3-pentanoic acid. α-Lipoic Acid is a white or off-white amorphous, hygroscopic powder having molecular weight 206.33 g/mol [8,9]. Structure of α- Lipoic Acid Shows in Fig No.2

Figure 1: Chemical structure of Allopurinol

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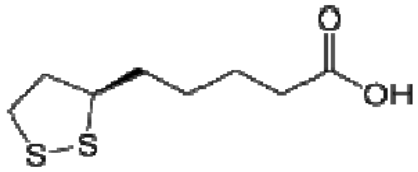

Figure 2: chemical structure of α-Lipoic acid

The review of literature regarding quantitative analysis of Allopurinol and α-Lipoic Acid revealed that no attempt was made to develop analytical methods for Allopurinol and α-Lipoic Acid. Some spectrometric methods and chromatographic methods have been reported for the estimation of the individual drugs [3, 8, 10 - 27]. The focus of the present study was to develop and validate a rapid, stable, specific, and economic spectroscopic method for the estimation of Allopurinol and α-Lipoic Acid in combination tablet.

MATERIALS AND METHODS

Allopurinol was purchased from Purvi chemical Pvt. Ltd and α-Lipoic Acid was gifted from Alembic Pharma.Methanol AR Grade (FINAR) use for method development.A double beam UV/Visible spectrophotometer (Shimadzu model 2450, Japan) with spectral width of 2 nm, 1 cm quartz cells was used to measure absorbance of all the solutions.

Standard stock solution for Allopurinol (ALO)

An accurately weighed quantity of Allopurinol (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of ALO (100 µg/ml). Sonicate for 10 mins if require.

Standard stock solution for α-Lipoic Acid (LA)

An accurately weighed quantity of α – Lipoic Acid (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration of LA (100 µg/ml).

Approach to AUC & Absorption correction method

In combination dosage form Allopurinol and α-Lipoic Acid ispresentin 1:1ratios, respectively.The absorption spectra of pure drug and their mixture were recorded between200-400 nm using methanol assolvent. The A0 LO was shows max. absorbance at 250 nm and L A shows max. absorbance at 328nm. LA has less Absorbance compare to Allopurinol for 10 to 50 ppm so in this method we consider area under curve at 310 nm to 390 nm in case of LA which found linear in given range of concentration and there is no interference of ALO in this region because it has no absorbance in this region.Allopurinol is estimated by absorption correction method at 250 nm. Overlain spectra of both drug shown in fig. 3 and linearity spectra for area under curve of LA shown in fig 4.

Calibration Curve for ALO & LA

The series consisted of five concentrations of standard ALO and LA solutions ranging from 10-50 µg/ml. The solutions were prepared by pipetting out 1ml, 2ml, 3ml, 4ml and 5ml Standard ALO and LA stock solution and transferred into a series of separate 10 ml volumetric flasks and volume was adjusted up to mark with methanol.

Estimation of ALO & LA in Tablet Dosage Form

Twenty 'ALUNO A tab' Tablets (containing 100 mg of Allopurinol and 100 mg of Lipoic Acid) were accurately weighed and ground to fine powder. An accurately weighed quantity equivalent to 50 mg of Allopurinol and 50 mg of Lipoic Acid from the formulation fine powder was transferred to 100 ml volumetric flask made up to the mark with the methanol. Sonicate for 15 mins. Filter Stock solution (500 µg/ml). Take 2 ml dilute up to 10 ml(100 µg/ml). From above solution take 3 ml dilute up to 10 ml (30 µg/ml). Note absorbance at 250 nm and take AUC at 310 to 390 nm.Calculate Allopurinol conc. Using

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absorption correction equation and Lipoic acid conc, using y=mx + c equation.

Validation of Method [28]

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 10-50 µg/mlfor both ALO and LA.

Precision

The precision of the developed method was assessed by analyzing standard solution containing three different concentrations 20, 30, 40 µg/ml for both ALO and LA. For Intraday precision three replicate (n=3) each on same day.For Interday Precision three replicate (n=3) for consecutive 3 days.These %RSD value was found to be less than ± 2.0 % indicated that the method is precise.

Accuracy

To demonstrate the accuracy of the proposed method, recovery studies were carried out by standard addition method. Solution of formulation in concentration 15 µg/ml for both ALO and LA was spiked with 50%, 100% and 150% concentration of standard for both ALO and LA (7.5, 15 25.5µg/ml) respectively. % recovery was then calculated by using regression equation.

Limit of detection

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOD was measured by using mathematical expressions given in section.The limit of detection (LOD) ofthe drug was calculated by using the following equations designated by International Conference on Harmonization(ICH) guideline:

$$LOD = 3.3 * \sigma/S$$

Where, σ = the standard deviation of the response
S = slope of the calibration curve.

Limit of quantification

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOQ was measured by using mathematical expressions given in section.The limit of quantification (LOQ) of the drug was calculated by using the following equations designated by International Conference on Harmonization (ICH) guideline:

$$LOQ = 10 * \sigma/S$$

Where, σ = the standard deviation of the response
S = slope of the calibration curve.

Robustness and Ruggedness

Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition, individually, the:

- Change in Stock Solution Preparation,
- Change in instrument (UV-Vis Spectrophotometer model 1800 and 2450)

Three replicates were made for the concentration (20, 30, 40µg/ml ALO & LA) with different stock solution preparation and the recording of absorbances was done on both the UV-Vis spectrophotometer. %RSD was calculated.

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Stock Solution Stability

The sample preparations were analyzed by UV at regular intervals for 72hrs as per test procedure.

RESULT AND DISCUSSION

Linearity

The Zero order spectra (Fig.3) showed line arabsorbance at 250 nmfor ALO (10-50 µg/ml) and (Fig.4) linear AUC at 310 to 390 nm for LA (10-50 µg/ml) with correlation coefficient (r^2) of 0.9998 and 0.9999 for ALO and LA, respectively. Linearity data for both drug shown in Table 1 and calibration curve graph and r^2value Showed in Fig 5 and 6.

Precision

Inter day and intraday precision data shown in Table 2 and 3 respectively. These% RSD value was found to beless than± 2.0indicatedthatthemethod is precise.

Accuracy

The % recover y values are tabulated in Table 4and Table 5. % recovery for ALO and LA by this method was found in the range of 101.18 to 101.61 % and 100.81 to 101.84 %, respectively. The value of % RSD within the limit indicated the methodise accurate and percentage recovery shows that thereis no interference from the expedients.

LOD and LOQ

The LO D for ALO and LA was conformed to be 0.16 µg/mland0.46 µg/ml, respectively. The LOQ for ALO and LA was conformed to be 0.5 µg/ml and 1.38 µg/ml, respectively.

Robustness and Ruggedness

The obtained Ruggedness and Robustness results are presented in table 6. The % R.S.D was found to be in range 0.11 – 0.66 % for ALO and 0.43 – 1.41 % for LA. No significant changes in the spectrums were observed, proving that the developed method is rugged and robust.

Assay Data of Marketed Formulation

The percent assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing ALO and LA. The % assay values are tabulated in Table 7.

Table 1: Calibration data for ALO and LA at 250 nm and 310 to 390 nm respectively. *(n=6)

Sr. No	Concentration (µg/ml)		Abs.* (250 nm) ±SD ALO	AUC* (310 to 390 nm) ±SD LA
	ALO	LA		
1	10	10	0.6819± 0.00359	0.1328± 0.00544
2	20	20	1.2878± 0.00215	0.2704± 0.00347
3	30	30	1.9101± 0.00622	0.4223± 0.00632
4	40	40	2.5085± 0.00416	0.5795± 0.00664
5	50	50	2.9928± 0.00513	0.7314± 0.00593

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Table 2: Intraday precision data for estimation of ALO and LA

PRECISION	Conc.	Allopurinol 250 nm	Lipoic Acid 310 to 390 nm
INTRADAY (n=3) Abs./AUC ±% RSD	20	1.3352 ±0.45	0.2727 ±1.06
	30	1.9411 ±0.57	0.4249 ±1.16
	40	2.6727 ±0.11	0.5736 ±1.10

Table 3: Interday precision data for estimation of ALO and LA

PRECISION	Conc.	Allopurinol 250 nm	Lipoic Acid 310 to 390 nm
INTERDAY (n=3) Abs./AUC ±% RSD	20	1.3362 ±0.62	0.2732 ± 1.41
	30	1.9414 ±0.68	0.4252 ± 1.19
	40	2.6717 ±0.25	0.5814 ± 1.04

Table 4: Recovery data of ALO*(n=3)

Conc of ALO from formulation (µg/ml)	Amt. of Std. ALO added (µg/ml)	Total Amt. of ALO (µg/ml)	Total Amt. of ALO found (µg/ml)* Mean ± SD	% Recovery*	% RSD ALO
15	7.5	22.5	22.7 ±0.08	101.27	0.35
15	15	30	30.48 ±0.06	101.61	0.20
15	22.5	37.5	37.94 ±0.17	101.18	0.44

Table 5: Recovery data of LA*(n=3)

Conc of LA from formulation (µg/ml)	Amount of Std.LA added (µg/ml)	Total Amt. of LA (µg/ml)	Total amount of LA found (µg/ml)* Mean ± SD	% Recovery* (n=3)	% RSD LA
15	7.5	22.5	22.68 ±0.32	100.81	1.37
15	15	30	30.47 ±0.19	101.56	0.65
15	22.5	37.5	38.14 ±0.31	101.84	0.81

Table 6: Robustness and Ruggedness data of ALO and LA

Condition	Conc. (µg/ml)	Different Instrument		Different Stock Solution Preparation	
		UV-2450	UV-1800	Stock-1	Stock-2
Allopurinol Mean (n=3) Abs. ± % RSD	20	1.3352 ±0.45	1.3361 ±0.43	1.3362 ±0.62	1.3419 ±0.42
	30	1.9411 ±0.57	1.9459 ±0.34	1.9413 ±0.66	1.9429 ±0.39
	40	2.6727 ±0.11	2.6782 ±0.39	2.6717 ±0.25	2.6762 ±0.40
Lipoic Acid Mean (n=3) AUC ± %RSD	20	0.2727 ±1.06	0.2710 ± 0.68	0.2732 ± 1.41	0.2736 ± 0.98
	30	0.4249 ±1.16	0.4246 ± 1.02	0.4252 ± 1.19	0.4262 ± 1.10
	40	0.5736 ±1.10	0.5814 ± 0.43	0.5814 ± 1.04	0.5783 ± 0.69

Table 7: Assay Result of Marketed Formulation

Formulation	Labeled Amount (mg)	% Assay
ALUNO A Tab	Allopurinol 100 mg	101.20 ± 0.58 %
	Lipoic Acid 100 mg	100.69 ± 1.73 %

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Table 8: Summary of Parameter

Sr. No.	Parameter	Allopurinol	Lipoic Acid
1	Linearity (µg/ml)	10 - 50	10 - 50
2	Correlation coefficient (r^2)	0.9998	0.9999
3	LOD (µg/ml) (n=10)	0.16	0.46
4	LOQ (µg/ml) (n=10)	0.5	1.38
5	Precision Intra-day (%RSD)(n=3) Inter-day (%RSD)(n=3)	 0.11 – 0.57 0.25 – 0.68	 1.06 – 1.16 1.04 – 1.41
6	Mean Recovery(%)	101.35 %	101.41 %
7	Assay(%)	101.20 ± 0.58 %	100.69 ± 1.73 %
8	Robustness and Ruggedness (%RSD)	0.11 – 0.66	0.43 – 1.41

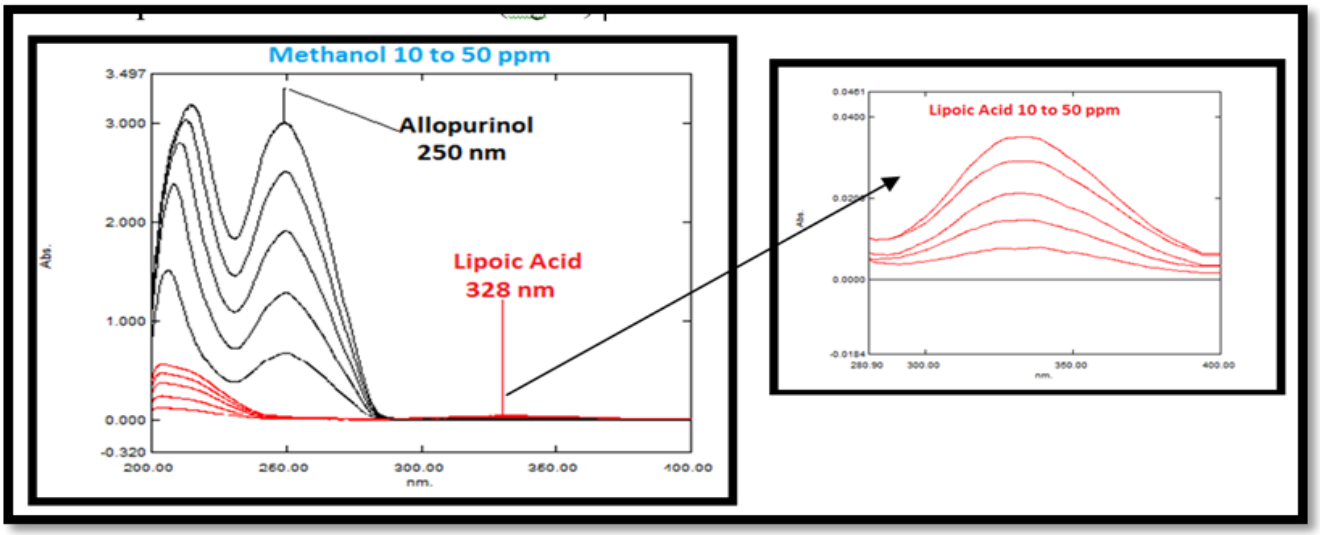

Figure 3: Overlain linear first order spectra of ALO (black) and LA (red) in 1:1 ratios

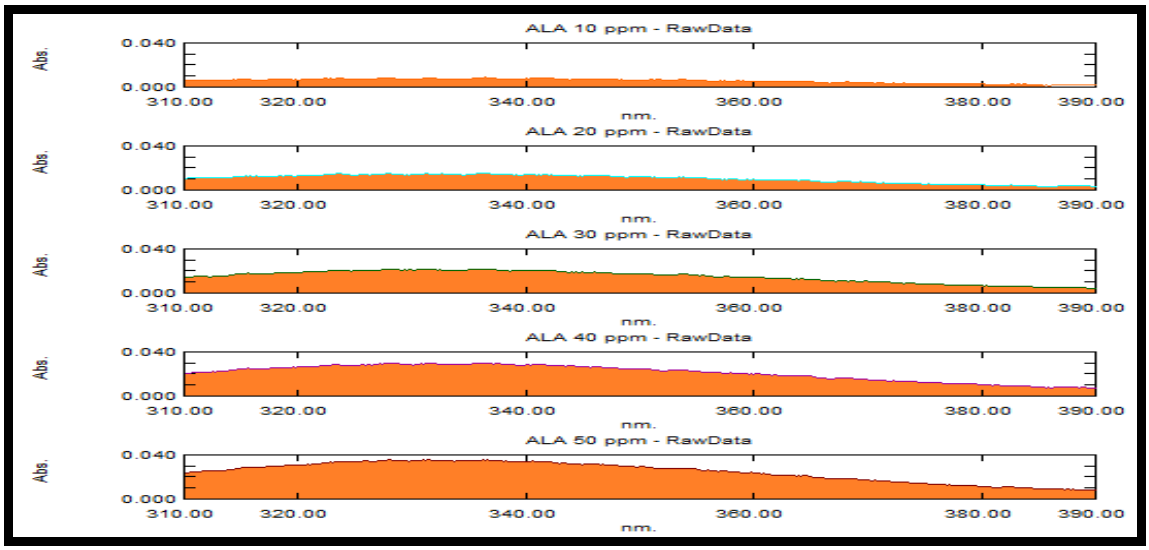

Figure 4: Area under curve of LA of concentration 10 to 50 ppm

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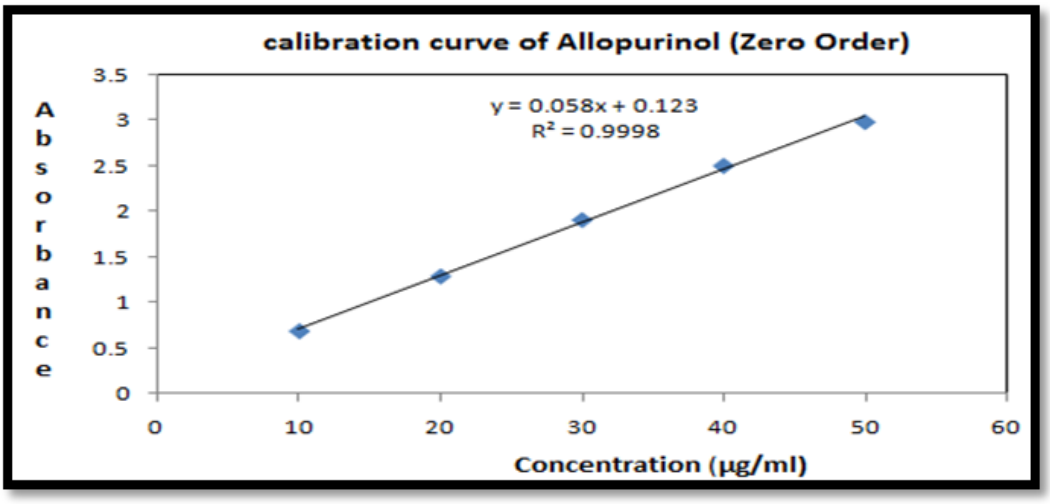

Figure 5: Calibration curve for ALO at 250 nm

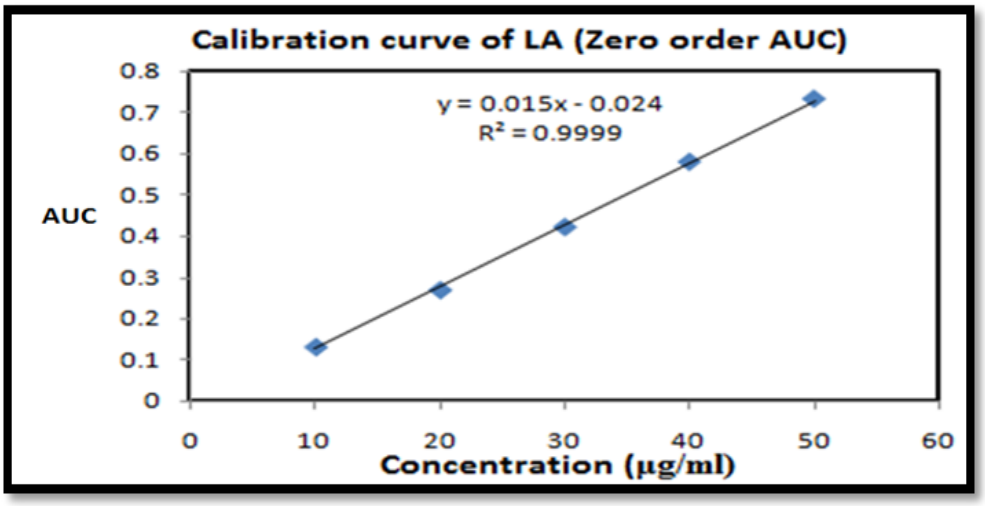

Figure 6: Calibration curve for AUC of LA at 310 to 390 nm

Summary Of Parametres

Summary of all the parameters given in table 8.

CONCLUSION

All the parameters for two substances met the criteria of the ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Ruggedness and Robustness of method was confirmed as no significant were observed on analysis by subjecting the method to slight change in the method condition.

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