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Research Article

DERIVATIZED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF GLUCOSAMINE AND IBUPROFEN IN TABLETS

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ABSTRACT

A selective, precise and derivatized HPTLC method has been developed for the simultaneous estimation of Glucosamine and Ibuprofen in tablet formulation. In this method standard and sample solutions of Glucosamine and Ibuprofen were applied on pre-coated silica gel $60F_{254}$ TLC plate, and developed using Propanol-Ethyl acetate-Ammonia-Water (4: 3: 2: 1 v/v), as mobile phase and derivatized using Iodine vapor. The drugs on the plate were scanned at 254 nm. The dynamic linearity range was 5-25 µg/spot for Glucosamine and 2-10 µg/spot for Ibuprofen. The method was validated for precision, recovery and reproducibility.

Key words: Simultaneous estimation, HPTLC, Glucosamine and Ibuprofen.

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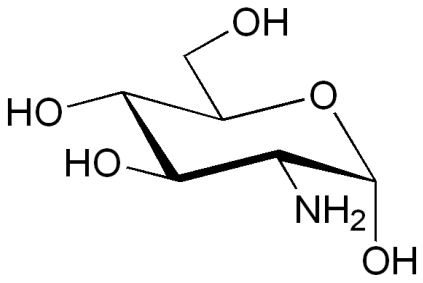
INTRODUCTION

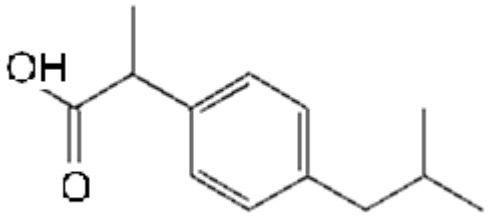
Glucosamine is an amino sugar, a prominent precursor in the biochemical synthesis of glycolated proteins and lipids, commonly used in the treatment of osteoarthritis. It is chemically (3R, 4R, 5S, 6R)-3-Amino-6-(hydroxymethoxy) oxane-2, 4, 5-triol[1]. Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) used for relief of symptoms of arthritis, primary dysmenorrhea, fever, and as an analgesic, especially where there is an inflammatory component. Ibuprofen is also known to have an antiplatelet effect. It is chemically known as (RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid[2].Literature survey revealed that various analytical methods like spectrophotometric [3], HPLC [4-12], HPTLC [13-15] and GC-MS [16] methods, have been reported for the determination of Glucosamine and Ibuprofen, individually and combination with some other drugs. No HPTLC method for simultaneous estimation of Glucosamine and Ibuprofen in combined dosage forms has so far been reported. The review of literature prompted us to develop an accurate, precise and derivatized simultaneous method for the estimation of Glucosamine and Ibuprofen in combined dosage forms.

Chemical Structure

Glucosamine



Ibuprofen



EXPERIMENTAL WORK

Chemicals and Equipment

PROCARTIL Tablet used for the formulation analysis contains Glucosamine (500 mg) and Ibuprofen (200mg) and it is manufactured and marketed by Centaur Pharma, Mumbai. Pure samples were procured from, Glucosamine – Culcruech pharma Ltd, Hyderabad and Ibuprofen – Aurobindo pharmaceuticals, Hyderabad. All the chemicals and reagents used were of analytical grade. A Camag HPTLC system comprising of Camag Linomat -5-applicator, Hamilton syringe, Camag twin trough chamber, Camag TLC scanner, and stationary phase pre coated with Silica gel $60F_{254}$ were used.

Preparation of Standard Solutions

The given standard Glucosamine 50mg was dissolved in Water and made-up to 10ml in a

volumetric flask, this solution used as working standard solution (5µg/1µl) for the analysis. The given standard Ibuprofen 100mg was dissolved in Methanol and made-up to 50ml in a volumetric flask, this solution used as working standard solution (2µg/1µl) for the analysis. Standard solutions having concentration ranging from 5 to 25 µg/ spot of Glucosamine and 2-10 µg/ spot of Ibuprofen were applied on TLC plates.

Analysis of Tablet Formulation

The given PROCARTIL twenty tablets were powdered using Pestle & Mortar to fine powder. From this, 250mg of powdered sample was extracted and dissolved in Methanol, centrifuged and the supernatant liquid was made-up to 50 ml in a volumetric flask with Methanol and filtered through Whattman filter paper no 41. This solution contains 5µg drug sample in 1µl Methanol, used as test solution for quantitative analysis of Glucosamine and Ibuprofen from PROCARTIL tablet. 4 µl of the test solution was applied on the pre-coated silica gel $60F_{254}$ plate and from the peak area obtained, the amount of Glucosamine and Ibuprofen in formulation was simultaneously calculated using the respective calibration graph. The amount obtained per tablet and percentage label claim are shown in Table 1.

Development of Chromatograms

Linearity

A series of 1µl, 2µl, 3µl, 4µl & 5µl standards of glucosamine and Ibuprofen solution were loaded in the 10 x 10 Silica gel $60F_{254}$ TLC plate using 100µl Hamilton syringe and Camag – Linomat -5 instrument.

LOD and LOQ

A working standard solution of 500ng/µl and 100ng/µl of glucosamine and ibuprofen were prepared in methanol. Series of 0.25µl, 0.5µl, 1µl, 2µl & 5µl Standard Glucosamine and ibuprofen solutions were loaded in the 10 x 10 Silica gel $60F_{254}$ TLC plate using 100µl Hamilton syringe and Camag – Linomat -5 instrument .

The TLC plates were pre washed with methanol and activated by keeping at 115° for about 30 min. The samples were spotted in the form of bands of width 5mm with 100 µl Hamilton syringe on the pre-coated silica gel $60F_{254}$ plate (10×10cm) and the slit dimension was kept at 15 min respectively. The mobile phase used was Propanol-Ethyl acetate-Ammonia-Water (4: 3: 2: 1 v/v) in chamber and the plate saturation time was 15 min, migration distance was allowed up to 80 mm, linear ascending development was carried out in (20×10cm) twin trough glass chamber. Subsequent to the development, TLC plates were dried in current of air and kept in photo documentation chamber. The images of developed plate were captured at white light, UV 254 nm and UV 366 nm using Camag – Reprostar -3 instrument. The developed plate was derivatized with iodine vapor and the images were done in white light using Camag – Reprostar -3 instrument. The derivatized plate was scanned at 254nm using Camag-TLC- scanner-3 instrument.

VALIDATION PARAMETERS

The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day assay precision, repeatability of measurement and repeatability of sample application.

Precision

Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days, and percentage relative standard deviation (%RSD) was calculated. The RSD was found to be less than 2 for both intra-day and inter-day precision. Repeatability of sample application was assessed by spotting 1 µl of drug solution, six times. From the peak areas, the percentage RSD was

determined. The complete validation parameters are shown in Table 2.

Recovery Studies

The recovery study was carried out at two levels, 50%, 100 %. To the powdered formulation, the standard drugs of Glucosamine and Ibuprofen were added at 50 % and 100 % levels, dilutions were made and analyzed by the method. The % recovery and % RSD were calculated and found to be within the limit, as listed in Table 3.

RESULTS AND DISCUSSION

During the stage of method development different mobile phases were tried and the mobile phase comprising of Propanol: Ethyl Acetate: Ammonia: Water in the proportion of (4:3:2:1 v/v) was confirmed. A good linear relationship was obtained over the concentration range 5-25 µg/spot of Glucosamine and 2-10 µg/spot for Ibuprofen respectively. The linear regression data showed a regression coefficient of 0.9995 for Glucosamine and 0.9994 for Ibuprofen. The LOD with signal/ noise ratio were found to be 500 ng and 200 ng/spot for Glucosamine and Ibuprofen respectively. The LOQ with signal/ noise ratio was found to be 1000 ng and 500 ng /spot for Glucosamine and Ibuprofen respectively. Assay results show excellent label claim of 99.2% for glucosamine and 98.5% for ibuprofen (table.1). The repeatability showed excellent % RSD less than 0.95 after six applications (table.2). The recovery was 100.96, 101% for glucosamine and 102,103.2%, for ibuprofen at 50% and 100% levels (table.3).

Table -1 RESULT OF ANALYSIS OF FORMULATION

Drug	Amount (mg/tablet)		% label claim*	S.D*
	Labeled	Found*		
Glucosamine	500	496.32	99.2	1.08
Ibuprofen	200	198.26	98.5	1.26

*An average value ± relative standard deviation of 5 observations.

TABLE -2 VALIDATION PARAMETERS

Parameters	Value	
	Glucosamine	Ibuprofen
R_f	0.25 ± 0.02	0.64 ± 0.04
Linearity (µg/ml)	5-25µg	2-10µg
Correlation co efficient	0.9995	0.9994
LOD (ng/spot)	500ng	200ng
LOQ (ng/spot)	1000ng	500ng
Precision (% RSD)		
Inter-day	0.83	1.18
Intra-day	0.51	0.78
Repeatability (% RSD)	0.65	0.95

R_f - resolution factor, RSD- relative standard deviation, LOD – limit of detection

LOQ – limit of quantification

TABLE -3 RECOVERY DATA

Level	Amount added (mg)		Amount found (mg)*		% Recovery*		% RSD*	
	glucosamine	ibuprofen	glucosamine	ibuprofen	glucosamine	ibuprofen	glucosamine	ibuprofen
50%	250	100	252.42	25.5	100.96	102	0.94	1.03
100%	500	200	505	51.6	101	103.2	1.02	1.33

*An average value ± relative standard deviation of 5 observations.

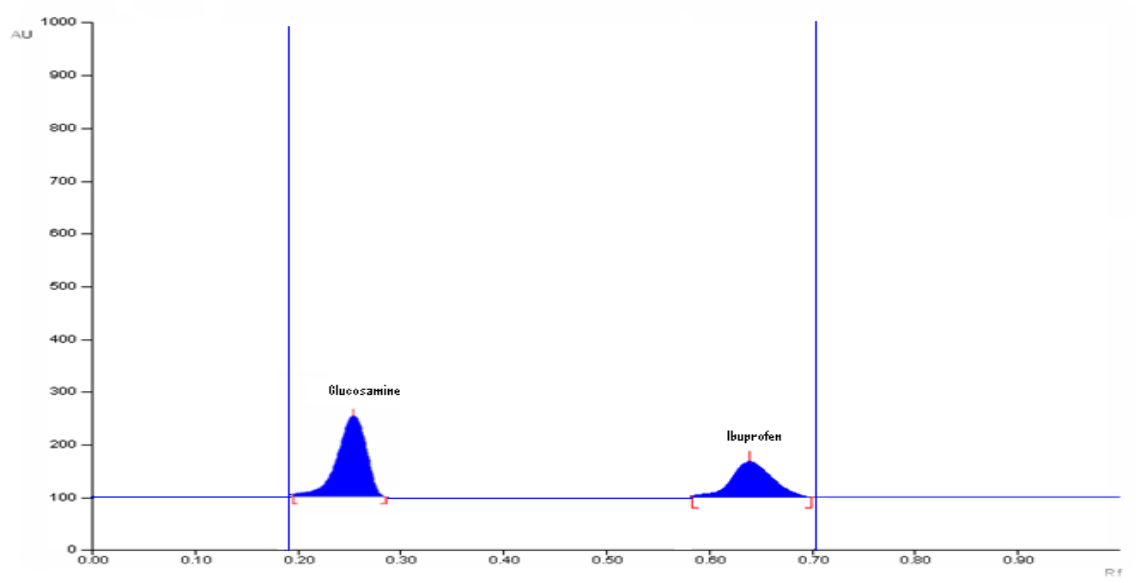


Fig 1: Chromatogram of Glucosamine and Ibuprofen.

Chromatogram showing resolution of Glucosamine (R_f = 0.25) and Ibuprofen (R_f = 0.64

CONCLUSION

The method passes all the validation parameter limits and proves to be selective, sensitive and precise. Hence the developed HPTLC method can be used for the simultaneous analysis of Glucosamine and Ibuprofen in bulk drugs and in pharmaceutical dosage forms without any interference from the excipients.

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