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Analysis of Chromium (III)Picolinate in Capsule dosage form by using Stability indicating HPTLC Method

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Abstract : Chromium (III) picolinate is used as a nutritional supplement and has been valuable effects on carbohydrate and lipid metabolism alleviating symptoms associated with diabetes. A chromium supplement produces beneficial results in reducing insulin sulfonylurea or metformin requirements. Hence, stability indicating HPTLC method was developed for estimation of Chromium picolinate in capsule formulation. The development of HPTLC method optimization on precoated silica gel 60 F_{254} aluminium plates of 20 cm x 20 cm, 250µm thickness. The mobile phase used was methanol: ethyl acetate6:4 (v/v). The densitometry detection was done at 264 nm. Also, the forced degradation studies were performed and method was validated with as per ICH guidelines. The Rf value obtained was 0.39 ±0.05.Linearity data for the calibration curve gave a good linear relationship over the concentration range of 100-600ng/spot for Chromium picolinate (correlation coefficient R^2=0.9997). The percent recovery of Chromium picolinate in marketed formulation was found in the range of 99.56%.Force degradation and validation of method was done as per ICH guidelines and the results are within the compliance limit.The developed HPTLC method gave good results for force degradation studies show that the method is stability indicating. Thus, this method can be used for routine analysis of Chromium picolinate and can be use for its future usage.
Keywords : HPTLC, Method validation, Stability indicating assay method.

Introduction

Chromium (III) picolinate (CP) chemically is 2-Pyridinecarboxylic acid chromium salt (Fig. 1).It is used as a dietary supplement which produces beneficial results in reducing insulin sulfonylurea or metformin requirements. Trivalent chromium is an essential activator of the insulin mediated reactions for glucose tolerance factor. By increasing its metabolism, chromium helps to maintain normal glucose level. Chromium increases insulin binding to cells, and activates insulin receptor kinase leading to enhanced insulin sensitivity [1-2].After executing a thorough literature survey, it is evident that the few methods areexisting for estimation

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of chromium picolinatein pharmaceutical dosage form such as Spectroscopic [2-3], HPLC [4-9], LC-MS[10], CE-IPC-MS [11], NMR[12]. Stability indicating HPTLC method is not reported so far for Chromium picolinate in the litreature. Hence, an aim has been made to develop and validate a stability indicating HPTLC method for analysis of Chromium (III) Picolinatein capsule dosage form.

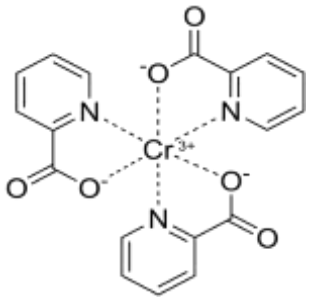

Fig 1: Structure of Chromium Picolinate

Materials and Methods-

Chemical and Reagents

Standard drug Chromium (III) picolinate was obtained from Sava Healthcare Limited, Pune as gift sample. Methanoland ethyl acetate were of AR grade and were purchased from Merck Chemicals, Mumbai, India.The capsules of Biotrex Nutraceuticals and Zenith nutrition (each capsule contains Chromium (III) picolinate800 mcg) is procured from the local market.

Instrumentation

The Camag TLC system consisted of Linomat V sample applicator (Camag, Muttenz, Switzerland). Camag TLC scanner III controlled by WinCATS software (1.4.4.6337) was used for sample application and quantitative estimation.Chromatography was performed on Merck silica gel 60 F_{254}precoatedaluminum TLC plates (20 cm × 20 cm with250 µm thickness), with methanol: ethyl acetate in the ratio of 6: 4v/v as mobile phase. Sampleswere applied as bands 6 mm long at 6 mm interval under a stream of nitrogen with a Camag 100 microlitre syringe(Hamilton, Bonded, Switzerland). The slit dimension was 5 mm × 0.45 mm. Ascending development to a distance of8 cm was performed in twin trough TLC developing chamber (Camag). Densitometric scanning was performed in absorbance mode at wavelength of 264 nm.

Preparations of stock solution of Chromium Picolinate

Chromium Picolinate was made soluble with the help of diluent, methanol: water (6:4). This diluent was used for preparing all the working solutions.

Stock solution of Chromium Picolinate (1000 µg/mL) was prepared by weighing 10mg of Chromium picolinate dissolving into a solution of methanol: water (6:4). The mixture was sonicated for 10 min to dissolve the drug and the solution was diluted up to 10 mL with same solution.

Preparation of marketed formulation solution

For checking the possibility of excipients interference in the formulation the assay was examined.Twenty capsules of (Biotrex and Zenith Nutrition) each capsule containing 800 mcg of Chromium picolinate were taken for determination of content of Chromium picolinate. Weighed 800 mcg equivalent powder of Chromium picolinate and dissolved in 5 mL of methanol, sonicated for 30 min with occasional shaking, and diluted to 10 mL with methanol. The resulting filtered solution 2 µL (160 ng/spot) was appliedon the TLC plate, which then developed and scanned.

Forced Degradation Study[13]

A stock solution containing 100 µg/µL of Chromium picolinate was prepared and used for forced degradation studies.As per the ICH Q1A (R2) guidelines the forced degradation studies were performed for acid, alkali, thermal, oxidative and photolytic stress conditions for Chromium picolinate.

Acid and base induced degradation studies

Refluxing the solution of drug in 0.1 N HCl at 60˚C for 2 hrs for acid decomposition studies. Under alkaline condition, refluxing the drug solution in 0.1 N NaOH at 60˚C for 2 hrs. The resulting solutions were applied to TLC plate in such a way that final concentration achieved was 100 ng/spot for both acid and base degradation studies.

Hydrogen peroxide induced degradation studies

Hydrogen peroxide induced degradation studies were performed in 3% hydrogen peroxide at room temperature for 3 hrs. Then the resultant solutions were applied to TLC plate (100 ng/spot)which then developed and scanned.

Thermal degradation studies

Place 30mg of Chromium picolinate in a petri dish and kept in hot air oven at 60˚C for 4 hrs. Samples were diluted with mobile phase and samples were spotted on TLC plate for analysis.

Photolytic degradation studies

Photolytic degradation was done by exposing the drug solutionto direct sunlight for 4 hrs, then transferred the amount in volumetric flask and dissolved withmobile phase.

Validation of the Method[14]

Validation of the developed HPTLC method was carried out with respect to ICH guidelines parameters.

Results and Discussion

Optimizationof method

HPTLC studies of the sample obtained during the stress testing of Chromium picolinate under different conditions using mobile phase.The method was optimized through the various trials of mobile phases until good resolution obtained for Chromium picolinate. Methanol: ethyl acetate6:4 (v/v)was finally considered as the mobile phase which resulted in sharp, well-defined peak of Chromium picolinate as shown in Fig. 2.

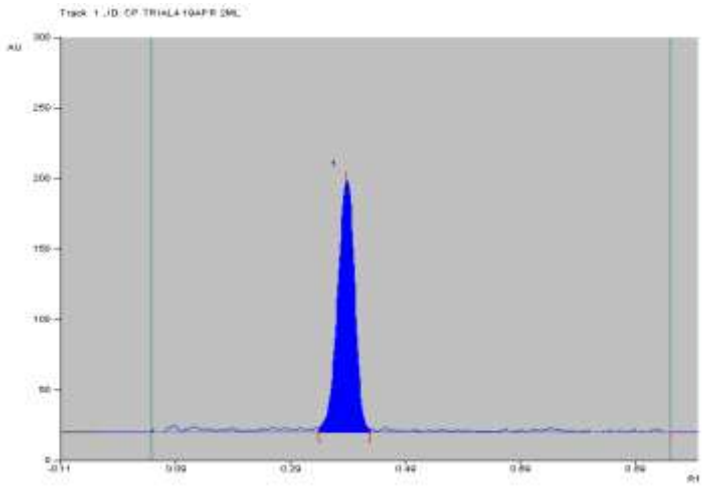

Fig. 2: Representative densitogram of Chromium Picolinate

Forced Degradation Study

The sample was degraded with acid, base, oxidative, thermal and photolytic showed well-separated spots of pure Chromium picolinate as well as some additional peaks at different Rf values.

Acid-Induced Degradation

For acid induced degradation studies of Chromium picolinate, it was found that around 12 % of the drug was degraded in 2 hr, forming a major degradation product at Rf0.09.

Base-Induced Degradation

It was found that around 17-18 % of the drug was degraded in base within 2 hr, forming a major degradation product at Rf0.07.

Hydrogen Peroxide-Induced Degradation

A densitogram of oxidative-degraded Chromium picolinate showed peak, at Rf 0.63 when around 10 % of the drug was degraded in 3 hr.

Thermal degradation studies

Chromium picolinate was found to be stable in thermal degradationstudies when it was kept in hot air oven at 60˚C for 4 hrs.

Photolytic degradation

Photolytic degradation of Chromium picolinatewas carried out. The drug was found to be stable in photolytic degradation.

Densitogram of Chromium picolinate in acid, base, H_2O_2, dry heat and light are as shown in Fig. 3**.**

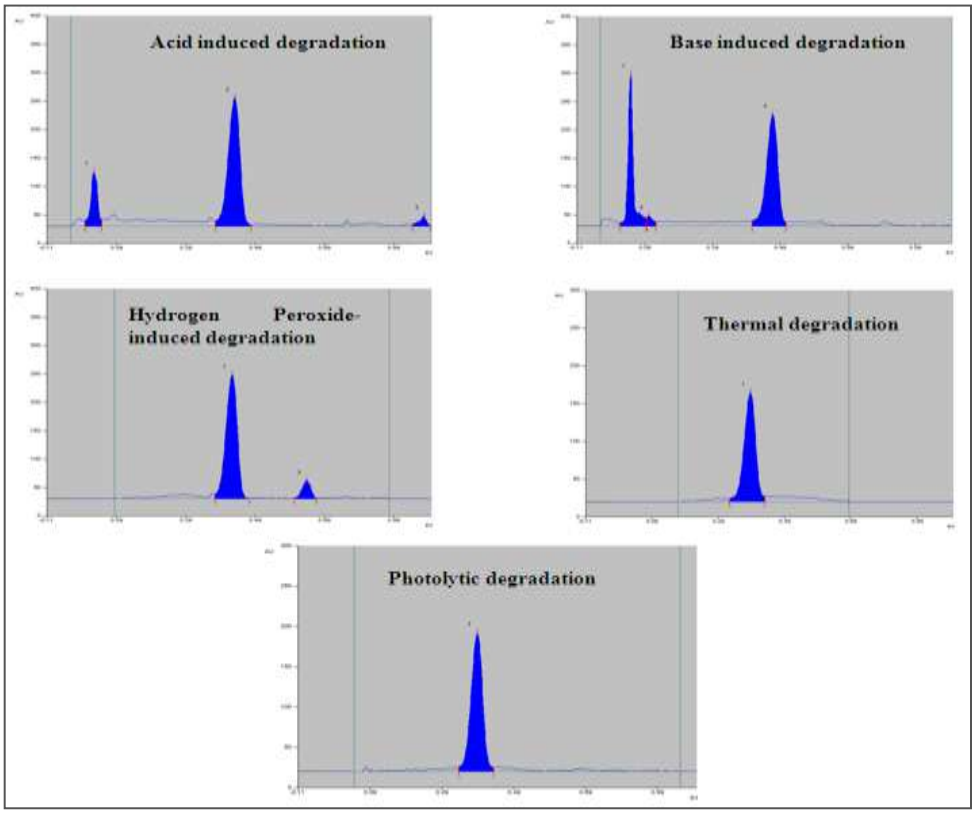

Fig.3: Densitograms of acid, base, oxidative, thermal and photolytic degradation of Chromium picolinate

Method validation

Linearity

Standard solutions of Chromium picolinate were prepared in theconcentration range of 100-600 ng /spot. Applied six times each concentration on the TLC plate.The plate was then developed.To determine the linearity of the method calibration curve was plotted between peak areas against the corresponding concentrations. The slope, intercept and correlation coefficient were determined from the calibration curve(Fig. 4).

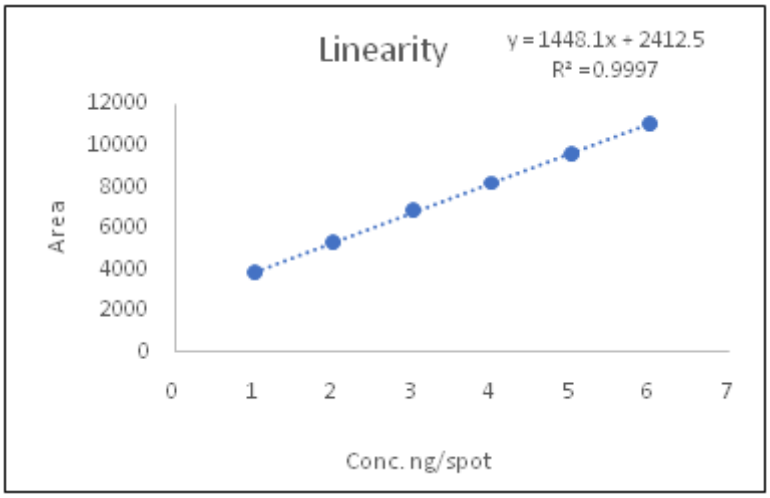

Fig. 4: Calibration curve of Chromium picolinate

Limit of quantification (LOQ) and Limit of detection (LOD)

Measuring the signal-to-noise ratio, LOD and LOQ were determined for Chromium picolinate by spotting a series lowest concentration of solutions until the S=N ratio 3 for LOD and 10 for LOQ. The LOD and LOQ were found to be 24.46ng/spot and74.14ng/spot, respectively.

Precision

Precision study of the method was carried out by performing an intraday and interday precision studies for Chromium picolinate. Using three different concentrations of Chromium picolinate200, 400, 600 ng/spotfor three times on the same day, intraday precision was performed and for inter-day precision of the method was checked by repeating studies on three different days.The outcomes weredetermined with %RSD.The results were reported in the Table 1.

Table 1: Precision studies (n = 3)

Concentration ng/spot	**Intraday precision**			**Intraday precision**		
	Avg. Area	**SD**	**%RSD**	**Avg. Area**	**SD**	**%RSD**
200	5390.1	73.44	1.36	5375.46	78.8	1.40
400	84823	26.29	0.31	8421.3	105.3	1.25
600	11061.3	96.57	0.87	11061.2	96.7	0.87

Table 2: Repeatability study (n=6)

Concentration	Area	Avg. Area ± SD	%RSD
200 ng/ spot	5286.15	5193.12 ± 28.88	0.55
	5292.55		
	5281.32		
	5189.41		
	5093.08		
	5190.25		

Repeatability

Repeatability of method was done by applying 200ng/spot of Chromium picolinate solution in six six times on a TLC plate and corresponding areas were recorded. The repeatability was express and calculated as % RSD. The results were given in Table 2.

Accuracy

Determining the recovery of theadded drug accuracy studies was carried out. This study was performed by applying the method to drug sample(Chromium picolinate capsules) to which know amount of Chromium picolinate standard powder corresponding to 80, 100, and 120%of label claim had been added (spike the sample), mixed, andthe powder was extracted and analyzed. Table 3 shows the recovery found for Chromium picolinate by accuracy study.

Table 3: Accuracy study (n=3)

Amount added (%)	Amount added (mcg)	Total amount (mcg)	Amount recovered (mcg)	% Recovery	% Mean Recovery ± SD
BiotrexNutraceuticals800 mcg/capsule					
80	640	1440	1426	99.03	99.38 ± 0.304
100	800	1600	1592	99.50	
120	960	1760	1753	99.60	
Zenith nutrition800 mcg/ capsule					
80	640	1440	1438	99.86	99.35 ± 0.450
100	800	1600	1584	99.00	
120	960	1760	1746	99.20	

Table 4: Robustness study

Conditions	Rf	Avg. Area ± SD	% RSD
Mobile phase composition ± 0.5 mL (v/v)			
Methanol: Ethyl Acetate (5.5: 4.5)	0.37	5903 ± 36.05	0.611
Methanol: Ethyl Acetate (6: 4)	0.39	5933 ± 36.11	0.609
Methanol: Ethyl Acetate (6.5: 3.5)	0.42	5881 ± 57.50	0.978
Development distance (± 0.5)			
7.5 cm	0.34	5898±10.06	0.170
8.0 cm	0.39	5886±33.30	0.566
8.5 cm	0.43	5933±49.15	0.828
Duration of saturation (± 5)			
25 min.	0.36	5809±20.5	0.353
30 min.	0.39	5823±39.57	0.680
35 min.	0.41	5610±36.55	0.651
Wavelength (± 2)			
262 nm	0.37	5820 ± 42.02	0.722
264 nm	0.39	5868 ± 60.00	1.022
266 nm	0.37	5902 ± 25.69	0.435

Robustness

Robustness studies were done by making small changes in the optimized condition.Each factor selected was changed at three levels (-1, 0, and + 1).Following are small deliberate variations in the chromatographic conditions like the change in mobile phase composition (± 0.1 mL), wavelength(± 2nm), duration of saturation (± 5 min) and developmentdistance (± 0.5 cm)and finding the corresponding Rf values and peak areas were recorded and % RSD was calculated (Table 4).

Assay of Marketed Capsule Formulation

Two different brands of Chromium picolinate capsules were analyzed using the proposed procedures.The content of Chromium picolinate was calculated andexpressed in percentage(Table 5).

Table 5: Analysis of Commercial Formulation (n=3)

Brand names of Chromium picolinate(800mcg/capsule)	**Rf**	**Amountfound(mcg) ± SD**	**(%) Content**
Biotrex Nutraceuticals	0.39	792.32 ±0.522	99.04
Zenith nutrition	0.39	795.84 ±1.406	99.48

Conclusion

Stability indicating HPTLC method has been developed for estimation of Chromium picolinate from capsule dosage form. Method validation was performed in accordance with ICH guidelines. This method could well separate the Chromium picolinate from their degradation products; therefore, it can be extended to study the degradation kinetics of Chromium picolinate in plasma and other biological fluids.

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