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ESTIMATION OF TRACE AMOUNTS OF CHROMIUM (III) IN VARIOUS MULTI-VITAMIN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

New specific, selective, simple & inexpensive spectroscopic method for estimating a trace amount of chromium (Cr^{+3}) from pharmaceutical formations was developed. The proposed method is based on the conversion of Cr^{+3} to Cr^{+6} by oxidation with a sodium hydroxide-hydrogen peroxide followed by the complexation of Cr^{+6} with 1.5 diphenylcarbazide (DPC) in a mineral acidic solution of pH 1.0±0.5. The pink-colored complex was estimated at 540 nm.The advantage of method is that it does not required preashing of the sample as it shown in the various method. Method was found to be linear in the range of 1-9 µg/ml. with a limit of detection is 0.166µg/ml and a limit of quantitation is 0.555 µg/ml. Method was found to be suitable for estimating Cr^{+3} species in various formulations. Satisfactory recovery from spiked samples of standard Cr^{+3} suggests no interference of any excipiants and diverse ions present in a formulation.

KEYWORDS

Chromium, Multi vitamin formulation

INTRODUCTION

Chromium (Cr) can exist in a various chemical valence states ranging from Cr^{+2} to Cr^{+6}, among which more stable chemical forms are Cr^{+3} and Cr^{+6}. Cr^{+3} are relatively non-toxic and are an essential nutrient in the human diet to maintain effective glucose, lipid and protein[3] metabolism. however , Cr^{+6} is primarily anthropogenic and as CrO_4^{-2} or $HCrO^{-4}$ can diffuse through cell membranes and oxidize biological molecules with toxic results[4].Cr^{+6} has been classified by the international agency for research on the cancer (IARC) as a group1 human carcinogen and by the US environmental protection agency (EPA)as a Group A inhalation carcinogen[5].

Chromium is one of the essential trace elements in multi-vitamin with multi-mineral pharmaceutical formulations that contain only Cr^{+3} either in the form of chromium chloride (inorganic source) or chromium picolinate and chromium polinicotinate (organic source). The methods for estimating Cr^{+3} from pharmaceutical formulation are atomic absorption spectroscopy (AAS)and graphite furnace AAS(GF-AAS).Other method reported in the literature for estimating Cr^{+6} are chemiluminescence's analysis, flame AAS, x-ray fluorescence spectroscopy (XRFS). various method for estimating Cr and total Cr include flow -injection Flame AAS from industrial effluents ;turbidimetry; ion chromatography ;anion-exchange

chromatography ; HPLC with diode array detection (HPLC-DAD) from environmental matrices fast protein liquid chromatography (FPLC), thermal lens spectrometry (TLS) , x-ray fluorescence .AAS from black tea and tea leaves food dietary products lung tissues , biological samples ,inductively coupled plasma-mass spectrometry (ICP-MS) from sea-water, ICP-atomic emission spectroscopy (ICP-AES) from human gallstone, neutron activation analysis (NAA) , from biological tissue surroundings , blood and milk .colorimetry[5] method from sshrimp feed[6],air[7,8] urine[7,9,10] and catgut suture[11].

One spectroscopic method has been reported for estimating Cr^{+3} from pharmaceutical formulation after ashing[1]. But this developed method was used for estimating Cr^{+3} from pharmaceutical formulation without ashing .Thus the primary objective of the present work is to develop , optimize and validate sensitive , selective ,precise , accurate , cost-effective , and simple spectroscopic method.

The proposed method was based on conversion of Cr^{+3} to Cr^{+6} by oxidation with a sodium hydroxide – hydrogen peroxide mixture[2], followed by estimating a pink-colored complex in a mineral acidic solution of pH 1.0±0.5 at 540 nm obtained by the reaction of Cr^{+6} with 1, 5 – diphenylcarbazide (DPC), which is considered to be one of the most sensitive and selective reaction for Cr^{+6} determination. The effect of pH, dye volume and stability of the colored complex, were studied for the method development.

MATERIALS AND METHODS

Apparatus

A Shimadzu UV – VIS spectrophotometer (model UV – 1700) with matched 1 cm silica cells is used for all spectral and absorbance measurements. The glassware use in each procedure rinsed thoroughly with double-distilled water and dried in dust-free air. What man filter paper No.42 was used to filter solutions of different formulations to separate them from the solvent immiscible formulation excipients.

Reagents

Double-distilled water and analytical-reagent grade chemicals were used. Standard solution of chromium were prepared by using chromium polynicotinate salt. A Cr^{+6} complexing reagent solution of 1, 5 diphenylcarbzide (DPC) (S.D Fine Chem. Ltd; Mumbai, India) was prepared by dissolving 0.25g of DPC in a 50 ml of acetone and stored in an amber colored bottle. This solution was discarded when it become slightly colored, and should not be used after 48 h of its preparation; it is always preferable to use it freshly prepared.

Preparation of standard solution for calibration curve

Chromium salt(chromium polynicotinate) equivalent to 1.0 mg of Cr^{+3} were subjected to oxidation by 20ml10%w/v sodium hydroxide and 15 ml of 6%w/v hydrogen peroxide added in order to obtain a stock solution of 50 µg/ml Cr^{+6}.From this stock solution , suitable aliquots were taken and add 0.2ml of DPC dye to each .allowed to stand for 5 min ,then 0.2ml of 10%w/v sulphuric acid was added two each .and volume was made up to 10 ml with distilled water to obtain the final concentration in the range of 1-9 µg/ml; the absorbance was measure at 540 nm.

Preparation of a sample solution

After twenty tablets/capsules were weighed and triturated, a powder equivalent to 1.0mg of Cr^{+3} was subjected to oxidation by using sodium hydroxide and hydrogen peroxide as per standard Conditions stated under: “preparation of standard solution for calibration curve” filter solution and volume was made to obtain 50 µg/ml of Cr^{+6} to Proceed further, as mention above. For soft gel sonicate sample for 30 min and then filter the solution and volume was made to obtain 50 µg/ml of Cr^{+6} to Proceed further, as mention above.

RESULTS AND DISCUSSIONS

Absorbance spectra

The absorption spectra of a Cr^{+6} DPC dye complex are graphically presented in Fig 1. As can be seen, the complex. Has maximum absorbance at 540nm

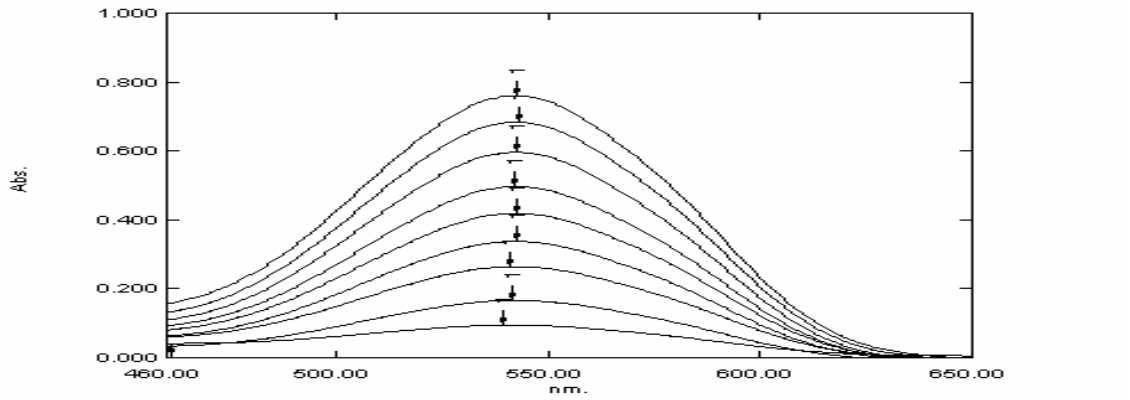


Fig 1

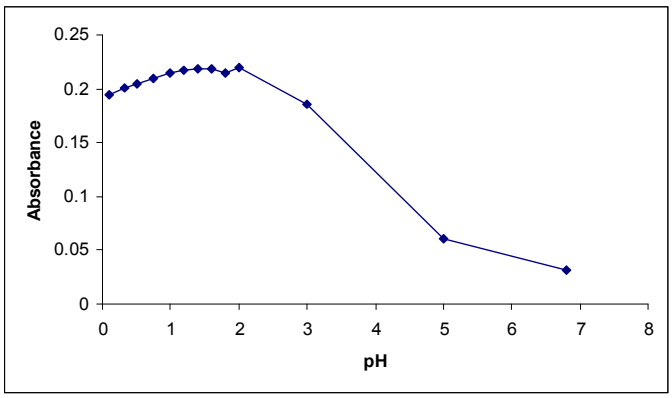
Visible spectra of Cr^{+6} :dye complex at 1 µg/ml(a),2 µg/ml(b),3 µg/ml(c),4 µg/ml(d),5 µg/ml(e),6 µg/ml(f),7 µg/ml(g),8 µg/ml(h),9 µg/ml concentration levels.

Effect of the pH

For stability of oxidized Cr^{+6} species an acidic environment is essential. Organic compounds and alkaline condition can cause Cr^{+6} reductions to Cr^{+3}, which will not form a complex with DPC dye. This was confirmed by maintaining different pH values (0.1, 0.330.5, 0.75, 1.0, 1.2,1.4,1.6,1.8, 3.0,5.0,6.8)of a solution containing the same concentration of oxidized species (5 µg/ml), the color intensity of the Cr^{+6} dye complex was drastically reduced as the pH increased , and because it was almost constant over the range of 1.0±5, it was selected as being optimum .The results for chromium polynicotinate salt used for method are shown in Fig.2.

Fig: 2

Effect of pH using chromium polynicotinate as a standard

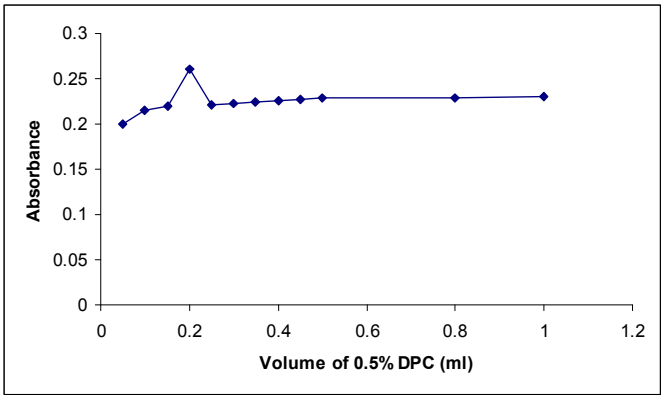


Effect of the dye volume

For evaluating the effects of the dye volume, different aliquots (0.05,0.1, 0.15, 0.2 0.25, 0.3, 0.35, 0.4, 0.45, 0.8, 1and 1.5) of 0.5% w/v DPC in acetone were taken for the same concentration of Cr^{+6} (5 µg/ml) .A dye volume of 0.2 ml of 0.5%w/v was found to be optimum for both salts, because at less volume the dye quantity was insufficient to form a Cr^{+6}:dye complex, and at higher volume of dye, the complex formation was interfered As present ed fig .3 , it showed the maximum absorbance at 0.2 ml of dye volume for chromium polynicotinate salt for the method.

Fig: 3

Effect of dye volume using chromium polynicotinate as a standard



Stability of colored complex

No significance change was observed in the absorbance for up to 4 h, but a significant reduction in absorbance during the 5 h, indicate stability of the colored complex to be four h.

Calibration curve sensitivity and validation

Under the recommended condition, the system obeys Beer s law over the concentration range of 1-9 µg/ml for chromium polynicotinate with an RSD of less than 1% at each concentration level. The low value of standard error and the coefficient of variation establish the precision of proposed method. Other parameter, like the molar absorptivity, regression equation, coefficient of determination (r^2), correlation coefficient(r), limit of detection (LoD), limit of quantitative. On (LoQ), etc .Are given in Table 1. The obtained correlation coefficient values are highly significants for method. Inter –day and intra –day RSD were also found to ascertain the precision of the developed method. As shown in (Table 2), RSD did not exceed 2% in any case. Recovery studies were carried out to ascertain the accuracy and precision of the methods by spiking the samples with known amounts of standard chromium salt to previously analysed the samples. Good recoveries of the analytes were attained (Table 3) with RSD less than 2.0%.

Table: 1
Optical characteristics and analytical data.

Parameters	Chromium polynicotinate
Absorbance maxima ,	540nm
Linearity range µg/ml	1 – 9
Coefficient of determination(r^2)	0.9991
Correlation coefficient(r)	0.9993
Regression equation (Y)[a]	
Slope(b)	0.084
Intercept(a)	0.0038
Limit of detection,LoD(µg/ml)	0.166
Limit of quatitation, LoQ(µg/ml)	0.555

a. y^a =a +bx, where x is concentration (µg/ml) .LoD=3σ/S .LoQ=10σ/S , where is a standard deviation of blank ,and S is slope of calibration.

Table: 2
Inter-day (n=3) and intra-day (n=6) precision and accuracy

Formulation	Labeled Cr claim / µg	Cr found[a] µg	labeled claim%	SD	RSD
Inter-day (n=3)	Tablet-	250	248.78	98.12	1.12 1.14
	Capsule-	200	199.43	99.13	1.97 1.99
	Soft- gel	200	200.22	100.87	1.44 1.45
Intra-day (n=6)	Tablet	250	249.49	99.22	1.27 1.28
	Capsule	200	199.55	99.31	1.42 1.43
	Soft-gel	200	200.26	101.06	1.74 1.72

a. Average of three and six determinations at two concentration level for inter-day and intra-day, respectively.

Table: 3
Recovery study of pharmaceutical formulations.

Formulation	Labeled Cr claim/µg	Standard Cr added/µg	Chromium found [a] /µg	Recovery %	RSD
Tablet	250	50	49.92	99.39	1.51
		100	99.63	98.58	1.71
Capsule	200	50	50.03	100.35	1.50
		100	100.38	100.39	1.68
Soft-gel	200	50	54.93	99.39	1.59
		100	99.66	99.08	1.09

a. Average of three determination

Effect of diverse ions on the determination of chromium

For studying the effect of diverse ions, a homogenous mixture containing various ions (like zinc, manganese, copper, iron silicon, selenium, iodide, molybdenum, vanadium ,etc) in different forms was prepared containing standard chromium salt (chromium polynicotinate) in a known quantity; the estimation was carried in the presence of interfering ions in order to judge whether the estimation is interfered or not. Good recovery for the added salt, as shown in Table 4, indicate no interference of diverse ions on the estimation of chromium.

Table :4
Effects of diverse ions on the estimation of chromium.

Ion	Added as	Quantity of ion
Zn^{+2}	$ZnSO_4$	15mg
Mn^{+2}	$MnSO_4$	2mg
Cu^{+2}	$CuSO_4$	2mg
Fe^{+2}	$FeSO_4$	15mg
Si^{+4}	SiO_2	2mg
Se^{+4}	$Na_2 SeO_4$	150 µg
Mo^{+6}	$(NH_4)_6 Mo_7 O_{24}$	75 µg
I^-	KI	150 µg
Cr^{+3}		200 µg
Cr^{+3} found		197.56 µg
Recovery,%		98.78 %

APPLICATION

The developed methods can be successfully applied for estimating Cr^{+3} from various pharmaceutical multi-vitamins with multi-mineral formulation such as tablets, capsules and soft gel without ashing. The analyses of various pharmaceutical formulations are enumerated in Table 5.

Table: 5
Result of the analysis of pharmaceutical formulations.

Formulation	Labeled claim /µg	Amount found /µg	labeled claim%
Co Ba Dex CZS Tablet	250	249.60	99.84
Benificieial capsule	200	199.22	99.61
Mims Soft-gel	200	199.58	99.79

REFERENCES

1. Jolly.M, Estimation of Trace amount of chromium (III) in multi-vitamin with Multi-Mineral Formulations, Analytical Sciences, 20: 1311 – 1315, (2004)
2. Svehla .G Vogal's Qualitative inorganic Analysis, Dorling Kindersley publisher 7th Edn: 135 – 138, (2006).
3. S.A ketz and H.salem. "The Biology & Envioronmental Chemistry of Chromium"1994.VCH New York, 43.
4. M.Stoeppler"Hazaddous Metal in the Environment Techniques and Instrumentation in Analytical Chemistry" 1992 Elsevier Amesterdem.373.
5. USEPA 600/8-83-014E "Health Assesment Document for Chromium", 1984, U S Envioronmental protection Agency Washington,D.C
6. N.Hara, Ind. Health, 1986, 24, 41.
7. S.divakaran,L.G.obaido, and I.P forster,J.Agric.Food Chem.,2002,50.464..
8. T.Dutkiewicz,J. Konczalik and M.Prezechera Acta Pol.,1969,26,165
9. W.matczak, T.rogaczewska, and J.Tsolnical, Med.Pr, 1986, 37,197
10. Y.Q.Li, V.H Zhao, Y.H.Cao,and M.Z.Shaho, Hua .Xi Yi Ke Da Xue Xue Bao. 1989, 20, 85.
11. G.H.Zhu, Zho. Yu Fang Yi Xue Za Zhi 1984, 18,173.