

A PAPER CHROMATOGRAPHIC METHOD FOR STABILITY
INDICATING ASSAY OF THIAMINE HYDROCHLORIDE
IN PHARMACEUTICAL PREPARATIONS

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Paper chromatographic separation of thiamine hydrochloride from its degradation products has been successfully carried out on Whatmann No. 1 chromatographic paper using n-butanol, acetic acid, acetone, water (45:20:10:25) as the developing system. For quantitative determination, the separated vitamin was eluted with acidic potassium chloride solution and determined spectrophotometrically at 250 nm. The recovery of thiamine hydrochloride was found to be satisfactory (S.D. = 0.1017 & C.V. = 1.0184). The method was successfully applied for the determination of thiamine hydrochloride in synthetic mixtures and in single and multicomponent pharmaceutical products with good precision and almost complete recoveries.

Thiamine as hydrochloride or nitrate, Vitamin B₁, is an essential nutritional or therapeutic components of numerous oral and injectable pharmaceutical preparations. It might undergo decomposition through oxidation or cleavage at the methylene group either due to entrensic formulation factors and/or the condition of storage. Several analytical procedures including, biological, physical and

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chemical methods have been proposed and applied for quantitative determination of thiamine⁽¹⁻⁴⁾. Biological assay methods are usually considered tedious and unreliable for routine analysis. The most widely used chemical method is the thiochrome method⁽⁵⁾, but close attention to details makes this method unsuitable for rapid control work⁽⁶⁾. In addition, it is interfered by the presence of other fluorescent compounds, and it is reported that, this method is unapplicable in the presence of certain drugs⁽⁷⁾.

Furthermore, it was observed during the course of the presented work that, most of the spots of the degradation products of thiamine hydrochloride separated on the paper chromatogram, similarly fluorescent as the spot of the intact vitamin, when sprayed with the alkaline ferricyanide reagent and visualized under ultraviolet lamp.

The direct ultraviolet spectrophotometric determination of thiamine, also, suffers from interference by the degradation products and other formulation ingredients which absorb ultraviolet at the wavelength at which thiamine is measured.

The specificity of the chemical methods could be improved through separation of thiamine from its degradation

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products and other interfering substances by the use of chromatographic techniques⁽⁷⁻²²⁾. Paper chromatography offers the most feasible technique for such purpose⁽¹³⁻²²⁾. It was aimed in the presented investigation to establish a paper chromatographic determination of thiamine hydrochloride in pharmaceutical preparations.

EXPERIMENTAL

Materials:

Thiamine hydrochloride, Riboflavine, Pyridoxin, Folic acid, Nicotinamide, Cyanocobalamine, L-Ascorbic acid, Cobalt sulphate, Zinc sulphate, Magnesium sulphate, Copper sulphate, Ferrous sulphate, Starch, Lactose, Sucrose, Phenol, Chloro-cresol, Methyl paraben, Glycerin, Propylene Glycol, and Polyethylene glycol 400. All these materials are either analytical or pharmacopoeial grades.

Methanol, Ethanol, Iso-propanol, n-Butanol, Amyl alcohol, Acetone, Benzene, Chloroform, Ethylacetate, Formic acid, Acetic acid, Hydrochloric acid, Potassium ferricyanide, Potassium chloride, and Sodium hydroxide, all are analytical grades.

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Whatmann No. 1 chromatographic papers.

Equipments:

- Ultraviolet Spectrophotometer, type Spektromon 203.
- Ultraviolet Lamp, type HPW-125W, 57202 E 17 Philips.
- Glass Chromatographic tanks, glass, 20x30x40 cm.

Procedures:

1- Separation of Thiamine Hydrochloride from Its

Degradation Products:

10 microliters of freshly prepared thiamine HCl solution in distilled water (1 mg./ml.), as standard solution; and solutions degraded in alkaline medium (prepared by mixing 1 ml of 1 mg/ml thiamine HCl solution and 4 ml of 0.5 N sod. hydroxide and stored for 2, 4, and 30 days at room temp.), were separately spotted on a chromatographic paper 20x30 cm. The spots were left to dry, the paper was then transferred to the ascending chromatographic tanks previously equilibrated with the developing solvent system (n-butanol, acetic acid, acetone, water; 45:20:10:2) for 24 hours. After the solvent front have reached about 20 cm. above the start line (7 hours), the chromatogram was removed and dried by a stream of hot air at 40°C. The chromatogram was then sprayed with the alkaline ferricyanide reagent (1.5 ml of

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1% pot. ferricyanide solution, 3 ml of 15% sod. hydroxide solution and 20 ml of dist. water), dried and visualized in the ultraviolet light. The fluorescent spots were defined and the R_f values of thiamine HCl and its degradation products were calculated.

2- Quantitative Analysis of Thiamine Hydrochloride

Along a Horizontal Line:

150 microliters of 1 mg/ml methanolic solution of thiamine hydrochloride sample, was applied along a horizontal line 10 cm. long in the middle part of 20x30 cm. chromatographic paper. Two reference spots, 10 microliters each, of standard thiamine hydrochloride solution (1 mg/ml) were spotted at both outersides of the paper, 2.5 cm. apart from the sample's line. The paper was dried, developed as previously mentioned, removed from the chromatographic tank and dried. The outer two strips of the chromatogram carrying the reference spots were separated, sprayed, dried and visualized in the ultraviolet light. The spots of thiamine hydrochloride were defined. The strips were aligned according to their original position on the paper and then two parallel lines were drawn defining the zone of thiamine HCl, which was then separated from the chromatogram and cut into small pieces.

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The pieces carrying thiamine HCl was placed into a glass stoppered tubes, eluted by shaking with 15 ml of 10% potassium chloride in 0.01 N hydrochloric acid, and then centrifuged. The supernatant liquid was measured spectrophotometrically at 250 nm, against a blank prepared by extracting an equal area of non-charged chromatographic paper developed under the same condition.

3- Application of the Developed Method for the Determination of Thiamine Hydrochloride in Synthetic Mixtures and Pharmaceutical Preparations:

I- Synthetic Mixtures:

A stock solution of thiamine HCl, 50 mg/ml, in dist. water was freshly prepared. From this solution, aliquots of 0.5 ml were separately transferred into a series of volumetric flasks, 25 ml, capacity. One flask was reserved to be used as a reference solution, and to each of the other flasks, an appropriate quantity of individual additive (Table 3) was added. The flasks were then completed to volume with methanol and shaken to dissolve as completely as possible. If necessary, the methanolic solution was clarified by centrifugation. The prepared solutions were then assayed as previously mentioned.

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II- Pharmaceutical Preparations:

A- Injectable Solutions:

An appropriate volume of the injectable preparation was withdrawn, transferred to a volumetric flask and diluted with methanol to give a final concentration of thiamine HCl of 1 ng/ml. The resulting solutions was then assayed as previously mentioned.

B- Tablets:

20 tablets were weighed and powdered. An accurately weighed quantity of the powder equivalent to 25 ng of thiamine hydrochloride, was transferred to 25 ml volumetric flask. This was then dissolved as completely as possible in methanol and the flask was completed up to the volume with methanol. The contents of the flask was then transferred to a centrifuge tube and centrifuged for 10 minutes. The clear supernatant solution was then assayed as previously described.

Recovery of Thiamine Hydrochloride from Pharmaceutical Preparations:

100 ng amounts of thiamine HCl, were added to an accurately weighed quantities of powdered tablets or an accurately measured volumes of injectable solutions, contained in

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an appropriate volumetric flask. Sufficient quantity of methanol was then added to dissolve and produce a final assay solution of 1 ng/ml. If necessary, the solution was clarified by centrifugation and assayed as previously described.

RESULTS AND DISCUSSIONS

As the authors were engaged with the stability studies on thiamine hydrochloride, an accurate and precise method of quantitative determination of the vitamin in degraded samples and in presence of various drug combinations and pharmaceutical excipients, was the first thing to be carefully considered. Chromatographic separation, in many instances, greatly facilitate the attainment of such requirements. Trials to apply the previously published paper chromatographic methods⁽¹³⁻²²⁾, for the quantitative separation of the vitamin from its degradation products, either failed to give adequate separation or showed poor reproducibility. So, it was decided to develop a paper chromatographic procedure giving maximum separation with good reproducibility.

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Various developing solvent systems have been tried using Whatmann No. 1 chromatographic papers differently conditioned. Table I shows the solvent systems which produce separation of thiamine HCl from its degradation products, compared on the basis of number of degradation spots could be separated as well as the absence of tailing in the separated spots. From this table, it is clearly obvious that, the most efficient solvent system in this concern was a mixture of: n-butanol, acetic acid, acetone, water (45:20:10:25). The time required for complete development of the chromatogram was seven hours.

Figure 1, shows the qualitative chromatographic pattern of thiamine HCl, degraded in alkaline medium. It is obvious from this figure that; up to six degradation products could be separated from solution of thiamine HCl degraded by sodium hydroxide at room temperature for 2 and 4 days. However, solutions degraded under these conditions for 10 and 30 days gave only four degradation spots. This might indicate that, the disappeared spots refer to intermediates which changed to the final degradation products upon storage for longer periods.

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The separation of thiamine hydrochloride from its degradation products along a horizontal line was satisfactorily affected up to a maximum load on the paper of, 150 μ g along a 10 cm. line. For quantitative determination, the zone of the intact vitamin was cut out and eluted with 15 ml of 10% KCL solution in 0.01 N HCL, then determined spectrophotometrically at λ max 250 nm, against a blank similarly prepared. A linear correlation between the concentration of thiamine HCL in the eluate and the extinction at λ max 250 nm was proved.

Table 2 clearly indicates that, the proposed method is of satisfactory precision and accuracy. The mean recovery of 10 determinations of thiamine HCL after chromatographic separation, was 99.81% with S.D. between the readings 0.10167 and the coefficient of variation equals 1.0184.

The method was applied for the determination of thiamine hydrochloride in presence of other vitamins, hormones, minerals and common excipients usually found in combination with the vitamin in pharmaceutical preparations. The quantities of the added materials approximate those usually occurring in pharmaceutical preparations. Table 3 indicates that, the developed method was highly satisfactory for the

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determination of thiamine in presence of most tested additives (percent recovery ranges from 98.9 to 101.22). However, in presence of copper and ferrous sulphate, low recoveries of thiamine was observed. Trials to overcome such interferences, by using chelating agents, were carried out, but negative results were obtained. The low recovery of the vitamin in such mixtures could be attributed to the decomposition of the vitamin in the presence of copper and ferrous ions, as revealed by the qualitative chromatographic separation. On the other hand, in presence of ascorbic acid, apparent very high results was observed, and this interference was overcome by mild oxidation of ascorbic acid to dehydroascorbic acid using 0.04 M solution of silver nitrate⁽²⁾, before carrying out the assay procedure.

The proposed method was further applied for the estimation of thiamine hydrochloride in various commercial pharmaceutical dosage forms, including tablets and injectable solutions, either contain vitamin B₁ singly or in combination with other vitamins. Table 4 shows that, the proposed method gives satisfactory results when applied for evaluating thiamine HCL content of the tested commercial products. The significant discrepancy between the found and claimed potency, observed in some products, might be attributed, at least in

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part, to partial decomposition of the vitamin in the product, as the assay procedures determine only the intact vitamin. Absence of interference of the compounding ingredients with the assay of the vitamin in these formulations, was proved by the almost complete recovery (98.92 - 102.4%) of the added thiamine hydrochloride to samples of such formulations.

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TABLE 1- Systems Tried for Chromatographic Separation of Thiamine Hydrochloride from its Degradation Products.

System	Ratio	Separation
1- Methanol, formic acid, water.	80:15:5	+ ve (tailed)
2- Isopropanol, Hydrochloric acid, water	65:20:15	+ ve (tailed)
3- n-Butanol, chloroform, ethylacetate.	25:50:25	+ ve (tailed)
4- n-Butanol, chloroform, benzene.	15:60:10:15	+ ve (tailed)
5- n-Butanol, chloroform, ethyl acetate, benzene.	15:60:15:10	+ ve (tailed)
6- Chloroform, Benzene, acetone, 10% urea in McIlv. buffer (pH 6.4).	70:15:15:10	+ ve
7- n-Butanol, acetic acid, water.	40:10:50	+ ve (tailed)
8- n-Butanol, acetic acid, amyl alc., water.	40:25:10:25	+++ ve
9- n-Butanol, acetic acid, acetone, water.	47:28:10:17	++ ve
10- n-Butanol, acetic acid, acetone, water.	45:20:10:25	++++ ve

TABLE 2: Recovery of 10 ugm. Thiamine Hydrochloride after Chromatographic Separation.

Expt.	Amount found	Deviation	
		in ugm	in %
1	10.225	+ 0.225	+ 2.25
2	10.102	+ 0.125	+ 1.25
3	9.938	- 0.062	- 0.62
4	10.020	+ 0.02	+ 0.20
5	9.897	- 0.103	- 1.03
6	9.938	- 0.062	- 0.62
7	9.750	- 0.250	- 2.50
8	9.816	- 0.184	- 1.84
9	10.102	+ 0.102	+ 1.02
10	10.020	+ 0.020	+ 0.20

Mean = 9.981 ; SD = 0.1017 ; C.V. = 1.0184

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TABLE 3: Determination of Thiamine Hydrochloride
in the Presence of Other Drugs and
Excipients.

Material	Amount Added (ng/5mg Vitamin B ₁)*	Amount of Vit. B ₁ (ng) Recovered*	Recovery (%)	S.D.	C.V.
Ethinyl estradiol	0.1	5.006	100.12	0.033	0.649
Methyl testosterone	2.5	5.040	100.8	0.081	1.613
Riboflavine	2.5	5.007	100.14	0.052	1.034
Pyridoxine HCl.	2.0	4.985	99.7	0.064	1.285
Folic Acid	2.0	5.012	100.04	0.041	0.818
Nicotinamide	10	4.977	99.54	0.065	1.316
Cyanocobalamin	0.001	4.943	98.86	0.087	1.757
Ascorbic Acid**	50	41.0	-----	-----	-----
CoSO ₄	1.55	4.97	99.40	0.0	0.0
Zn SO ₄	3.0	5.00	100.0	0.048	0.969
Mg SO ₄	5.0	5.059	101.18	0.031	0.618
Cu SO ₄ **	4.5	3.0	60	-----	-----
Fe SO ₄ **	100	1.711	34.22	-----	-----
Starch	50	4.960	99.20	0.108	2.167
Lactose	10	5.023	100.46	0.07	1.394
Sucrose	50	5.009	100.18	0.038	0.762
Alcohol	150	5.026	100.52	0.015	0.307
Glycerin	250	4.930	98.90	0.0	0.0
Phenol	5	5.046	100.92	0.088	1.737
Chlorocresol	1	5.031	100.62	0.014	0.028
Methyl paraben	0.5	5.023	100.46	0.166	3.294
Propylene Glycol	400	5.061	101.22	0.10	1.984
PEG 400	100	5.053	101.06	0.112	2.20

* Average of three Determinations
** Interfering Materials.

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TABLE 4- Application of the Assay Procedure for the Determination of Thiamine Hydrochloride in Pharmaceutical Dosage forms.

Formulation	Amount of Vitamin B ₁ * (ng. per ml. or per Tab.)				Recovery of 100 ng of Vitamin B ₁ * added		
	Claimed	Found	S.D.	C.V.	%	S.D.	C.V.
A- Ampouls:							
I	100	111.06	0.034	0.618	101.14	0.0	0.0
II	100	90.4	0.0	0.0	98.92	0.0196	0.207
III	100	101.0	0.0519	1.029	100.06	0.019	0.189
B- Vials:							
I	50	35.2	0.173	0.492	101.94	0.0289	0.331
II	100	81.64	0.035	0.857	102.40	0.041	0.446
C- Tablets:							
I	15	15.249	0.026	0.511	100.42	0.048	0.473
II	20	25.652	0.065	1.005	100.82	0.058	0.509
III	100	96.7	0.064	1.314	100.7	0.063	0.633
IV	250	242.85	0.044	0.905	101.96	0.052	0.527

* Average of three determinations.

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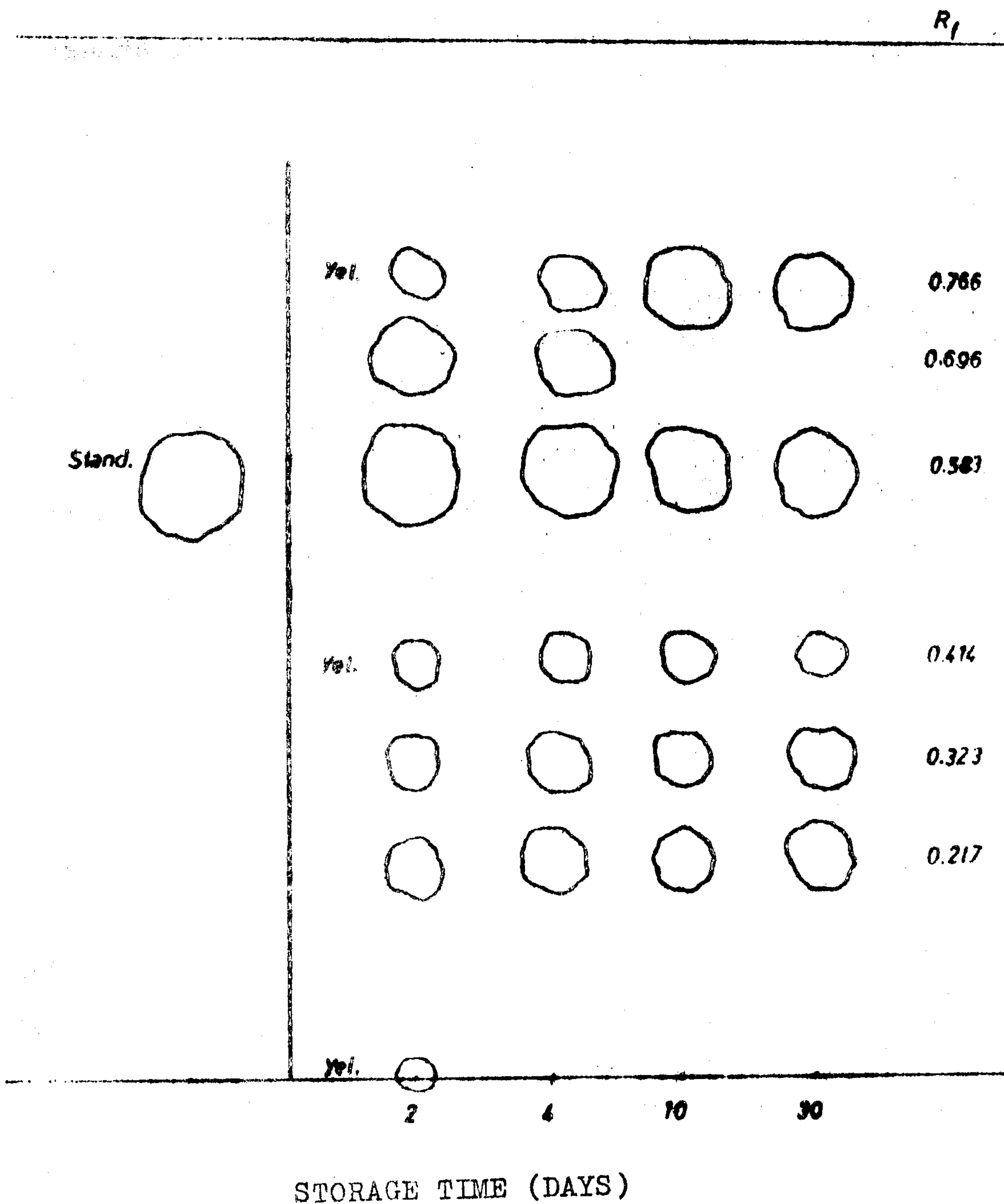


Fig. I: Schematic Chromatogram of the Degradation Products of Thiamine Hydrochloride in 0.4 N NaOH.

Key: Stand. = Standard Thiamine Hydrochloride; and Yel. = Yellowish Spot.

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پایه علمی و تحقیقاتی
روش کروماتوگرافی کاغذی برای پایداری
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طريقة كروماتوجرافيا ورقية
للتقييم المعنى بثبات ايد روكلوريد الثيامين
في المستحضرات الصيدلانية

على سينا - السيد على ابراهيم - حدى عبدالستار الصردى - سوزان شوقى

تم بنجاح عملية فصل ايد روكلوريد الثيامين (فيتامين ب₁) من نواتج
تحليله باستعمال كروماتوجرافيا الورق على ورق واشمان رقم واحد
باستعمال نظام مذيب للفصل مكون من مخلوط من البيسوتانول
المعادى وحمض الخليك والاسيتون والماء بنسبة ٤٥ : ٢٠ : ١٠ : ٢٥
هكذا وللتقييم الكمي للثيامين فيتم استخلاصه من موضع
على ورق الكروماتوجرافى بعد اتمام الفصل باستعمال مطول
حمضى من كلوريد البوتاسيوم ثم يتم قياسه بعد ذلك باستعمال مطياف
بعد موجته قدرها ٢٥٠ نى

وتد ثبت ان مقدار الاسترجاع للثيامين باستعمال هذه الطريقة مرضى حيث
وجد ان مقدار الحيود القياسى يساوى ١٠١٧ ر. ومعدل الاختلاف يساوى
١٨٤ ر.

وقد تم تطبيق هذه الطريقة بنجاح فى التقييم الكمي لايد روكلوريد
الثيامين فى مختبرات مختصة بالمعمل وكذلك فى المستحضرات الصيدلانية
المسوقة التى تحتوى على ايد روكلوريد الثيامين منفردا او مع غيره من
الأدوية وكانت النتائج دقيقة وثابتة ومعدل استرجاع كامل للدواء.