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RESEARCH ARTICLE

QUALITY EVALUATION OF PHARMACEUTICAL HUMAN SERUM ALBUMIN PREPARATIONS AVAILABLE IN PHARMACIES IN ADEN GOVERNORATE - YEMEN

Ahmed Thabet Ahmed Al-Sarhe[1,*] and Mutea'a Edrous Saeed[2]

[1] *Dept. of Chemistry, Faculty of Science, University of Aden, Yemen*
[2] *Dept. of Chemistry, Faculty of Education - Saber, University of Lahij, Yemen*

***Corresponding author: Ahmed Thabet Ahmed Al-Sarhe; E-mail: ahmedbiochem@gmail.com**

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Abstract

Human serum albumin(HAS) is important for the body, as it performs a set of functions such as maintaining osmotic pressure inside cells, transporting drugs and ions, and others. The fraudulent process of such preparations may lead to the deterioration of the patient's health condition and sometimes death. In the current study, two types of albumin preparations that were not authorized by the Yemeni ministry of health and entered the country through smuggling were studied. The results of the study proved that these preparations were exposed to high temperatures, so that denaturation of HSA, in addition to the oxidation process of the substance N-acetyl-tryptophan. The reverse phase (RP) high-performance liquid chromatography (HPLC) method to assess HSA, a gradient elution (a combination of acetonitrile/water, supplemented with 0.1% (v/v) trifluoroacetic acid) was used to separate samples on a C4 (n-butyl-coated silica) column. Two main peaks were observed at 4.970 and 10.850 min, representing the stabilizer N-acetyl-tryptophan (N-Ac-Trp) and HSA respectively. Validation of the method demonstrated that HSA can be determined in an accurate and precise manner, in a range between 0.1 and 5g/ml, without the interference of matrix ingredients. The limit of detection (LOD) and lower limit of quantification (LLOQ) values were 0.23 and 0.72 g /ml, respectively. The results of the study proved that these preparations do not meet the quality specifications of the World Health Organization, in addition to exposure to temperatures and bad storage leading to oxidation. The results of the analysis of all samples were less than the permissible limit because each sample must contain 10 grams per 50 ml.

Keywords: Human albumin, HPLC, Pharmacies, Aden.

Introduction

albumins are globular proteins commonly found in blood plasma, egg white, milk, and plants [1-4]. Serum albumin is the most abundant protein in the blood plasma of all vertebrates [5]. It is synthesized in the liver and matures in the endoplasmic reticulum and golgi bodies before being secreted from the hepatocytes [5,6].

(HSA) has a plasma concentration of 35-50mg/ml [6,7], an approximate half-life of 19 days, and it is present in both extravascular and intravascular spaces [7,8]. Albumin performs a variety of essential functions. It regulates of oncotic pressure and pH of the blood [5]. It also binds and transports various bioactive molecules, including proteins, fatty acids, hormones, amino acids, drugs, nutrients, and metal ions [6,9].

HSA is made up of a single chain of 585 amino acids. Its secondary structure is highly flexible, characterized by 67% α *helix* and 17 disulfide bridges with 6 turns that act as cross-linkers for the three homologous domains [5].

HSA is clinically used in hemorrhagic shock due to excessive blood loss, hypovolemia, and hypoproteinaemia [10,11]. In addition, purified HSA is commonly used in eukaryotic cell culture practices [12,13].

Classically, HSA was commercially produced by fractionating human plasma [14]. However, human plasma always has a limited supply. In addition, inconsistencies in the quality of the raw material from different sources and other contamination issues lead to

variations in the quality and quantity of the final purified protein.

The aromatic amino acids, N-acetyl-tryptophan, are included in the composition of albumin as a stabilizer, and these acids are sensitive to the oxidation process. For this reason, N-Acetyl-Tryptophan is added to rHSA 20% during manufacturing to prevent the oxidation process. But when exposed to high temperatures or poor storage, the substance N-Acetyl-Tryptophan is oxidized which leads to the degradation of albumin [15].

To the best of our knowledge, there have been no previous studies carried out for evaluation of albumin pharmaceutical preparations HSA that were not authorized by the Yemeni ministry of public health and entered the country illegally.

Significance of the Study:

1- Verification of quality specifications for unauthorized intravenous albumin preparations from the General Authority for Medicines located in Aden Governorate pharmacies.

2- Comparison between the results obtained from this study and the permissible limits in the international pharmacopeia.

3- Contribution to spreading health awareness about the dangers of smuggled drugs of unknown origin and composition, and introducing reliable sources to obtain information related to them from the Food and Drug Authority

4- Urging those in the Yemeni High Authority for Medicines and Medical Supplies to activate the authority's role in the field of follow-up, control, and inspection of smuggled and adulterated drugs.

Storage

1- Albumin (Human) 25% may be stored for 36 months at +"2 C^0 to +25"the date of manufacture.

2- Store in the original container to protect from light.

3- Do not freeze.

4- Do not use it after the expiration date.

Sample Collection

Samples were collected from pharmacies in Aden governorate during the period from 25-3 to 3-4 -2022. It was found that there were two types of albumen preparations available in most pharmacies, and to differentiate between these preparations, we symbolize one of them with the symbol "A" and the second with the symbol 'B".

Chemicals and Reagents

1- Human serum albumin 20% was purchased from the pharmacies in Aden city.

2- Ultrapure water (level 1+)

3- HPLC-grade acetonitrile (99.9%) conforming to the European Pharmacopoeia obtained from alpha chemical.

4- HPLC-grade trifluoroacetic acid (TFA 99.5%)

5- Isopropanol and methanol 99.9% were obtained from alpha chemicals.

All other chemicals and reagents were HPLC grade. All solvents were filtered through 0.45 um (pore size) filters (Millipore) and degassed [16].

Equipment

HPLC (produced by the Japanese company JASCO) a model LC-NET, equipped with a detector HPLC-Model LC-NET (produced by the Japanese company JASCO), is connected to a detector that contains special programs for analysis. A VYDAC 214TP C4 Column (250 mm × 4.6 mm), with a pore size of 300 Å. The large pores of the 300 Å silica allow polypeptide molecules to have complete access to the interior area of the silica pores [16].

Chromatographic Conditions

A gradient elution was applied for 20 min, with mobile phase A being 0.1% TFA (v/v) aqueous solution and mobile phase B being 0.1% TFA (v/v) in 100% acetonitrile (ACN). Both liquids were filtered through a 0.45 um filter before use. The gradient, setup is shown in Table 1 [15]. The autosampler was chilled to 4± C. Eluent was pumped at a flow rate of 1 ml /min, the injection volume was 20 ml, and the column department was heated to 40± C^0 and the detection wavelength was set to 280 ±2 nm.

Validity of Analysis Method

Linearity

A calibration curve is drawn for the study subject through the preparation of six stander solutions of HAS in the range (of 0.1 to 5g/ml) and injected into the HPLC according to the data shown in (Table 2)

The calibration curve is shown in Figure1, a linear response was obtained with a correlation rate of 0.998

And above the following linear equation: Y=7000000x-1183.4

Table 1: Gradient elution scheme for chromatographic separation of HSA .

Time (min)	% Mobile phase A	% Mobile phase B
0	80	**20**
5	60	**40**
8	55	**45**
10	40	**60**
11	0	**100**
13	0	**100**
14	80	**20**
20	80	**20**

Table 2: Peak areas for different concentrations of albumin stander solution and related information.

NO	Concentration g/ml	Area (µV*sec)	flow rate	Wavelength Weight	Temperature	RT	Mobil phase
1	5	35677775	1ml/mit	280nm	2 – 8C	10.850	A: 0.1% TFA (v/v) aqueous solution B: 0.1% TFA (v/v) in 99.9% Acetonitrile
2	2.5	177888887					
3	1	7179506					
4	0.7	5109658					
5	0.5	3551797					
6	0.1	611548					

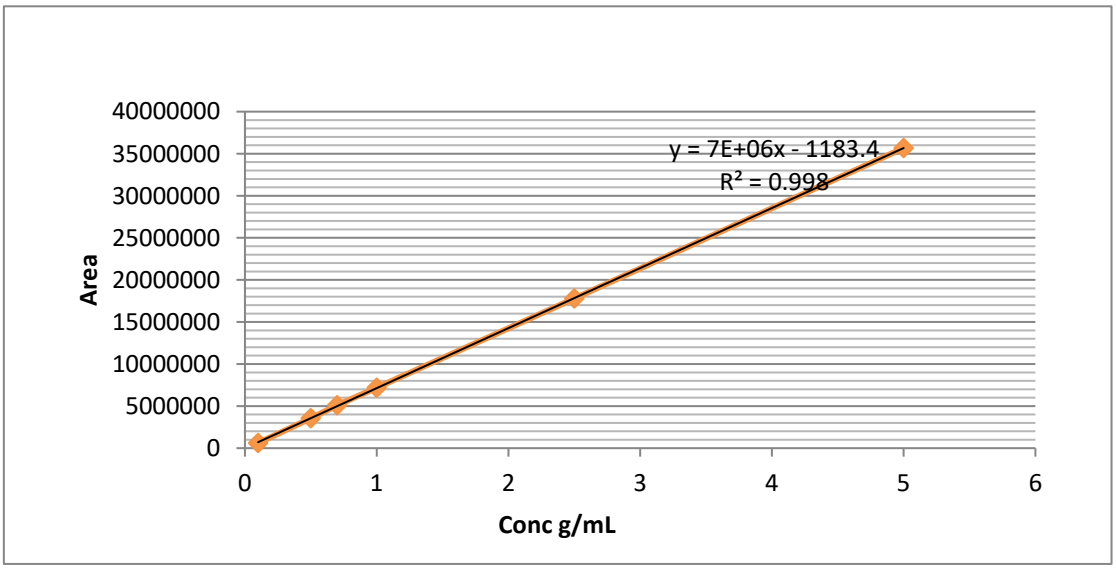

Fig. 1: Standard curve of HSA, with a concentration range between 0.1-5 g/ml

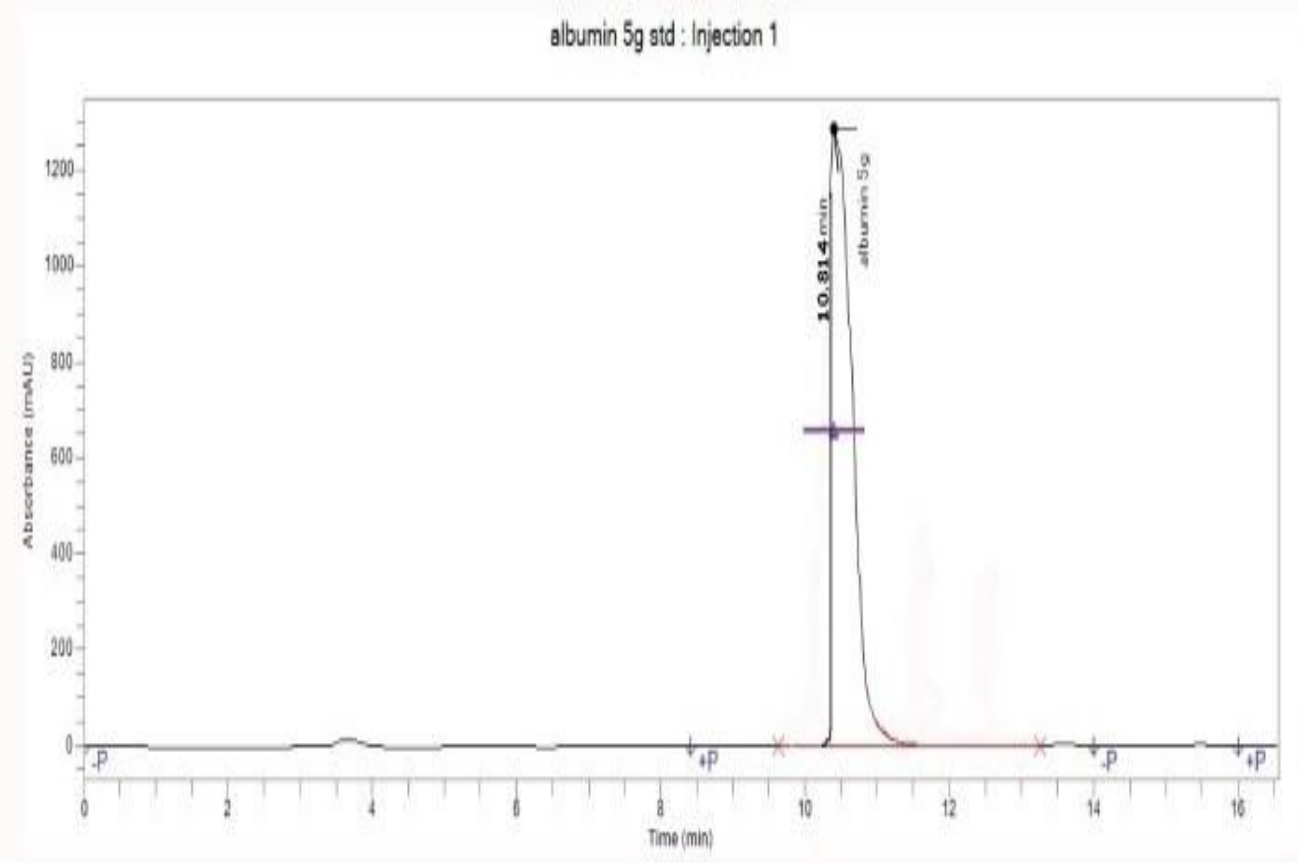

Fig. 2: Chromatographic analysis of the stander HSA-5g/ml cons

Accuracy

Accuracy should be established across the specified range of the analytical procedure as stated in the ICH Q2(R1) guidelines [17]. As shown in Table 4, the recovery percent of measured contents of spiked HSA to the standard

Table 3: Recovery value obtained from three samples prepared from standard.

Concentration tested (g/ml)	Calc. concentration (g/ml)	Recovery (%)	%RSD
5 g/ml	5.09	101.8	0.24
0.5 g/ml	0.49	98	0.33

Precision

Intra-day variability (repeatability) was determined by analyzing two HAS concentrations (0.5 g/ ml) and (2 g/ ml), which were injected 3 times respectively. Data are presented in Table 4, showing an RSD of 0.33% (0.5 g /ml) and 0.14% (2 g /ml) respectively.

Table 4: Intra-day variation (repeatability) assessed using two different HSA concentrations

Run/AUC	0.5 g/ml	2 g/ml
1	3,497,787.5	**13996954.6**
2	3,497,807.9	**13996983.1**
3	3,497,597.5	**13996942.2**
Average	3,497,730.9	**13996959.9**
SD	116	**20.79**
% RSD	0.0033%	**0.00014%**

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD of the HSA was determined by preparing a set of solutions with low concentrations

of the two stander solutions (0.5, 2g/ml) that are expected to produce a response that is 3-10 times the baseline noise. has been determining LOD and LOQ values, including calculation of the signal-to-noise ratio and the slope method which was used in the present study. The SD of the analytical response (or y-intercept) and the slope of the linear regression curve were applied in the respective mathematical formulas, yielding LOD

(3.3 ×SD/S) and LOQ (10 × SD/S) Values of 0.33 and 0.72 g /ml

detection and quantitation of these HAS at low concentration levels.

Results and Discussion

Peak Identification

The first peak (Rt 4.8): is for a substance of the N-Acetyl-Tryptophanate that is added to HSA 20% solution, to protect them from the oxidation process. And The second peak (Rt 10.850): is for HSA.

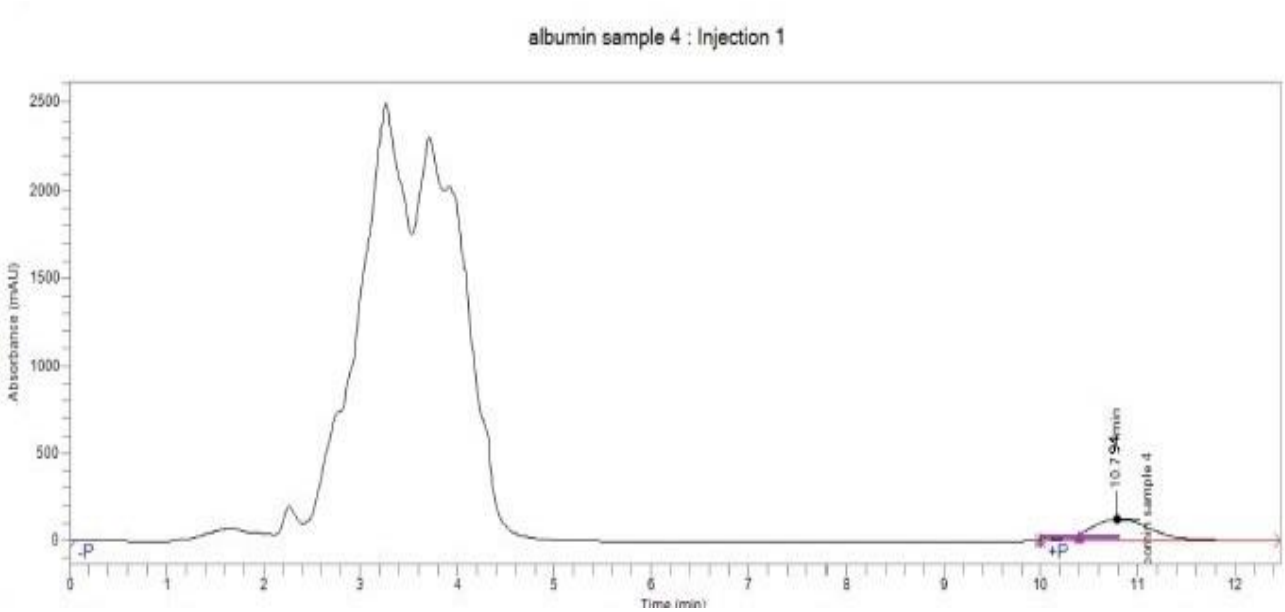

Fig. 3: Chromatogram of the HSA-sample A.

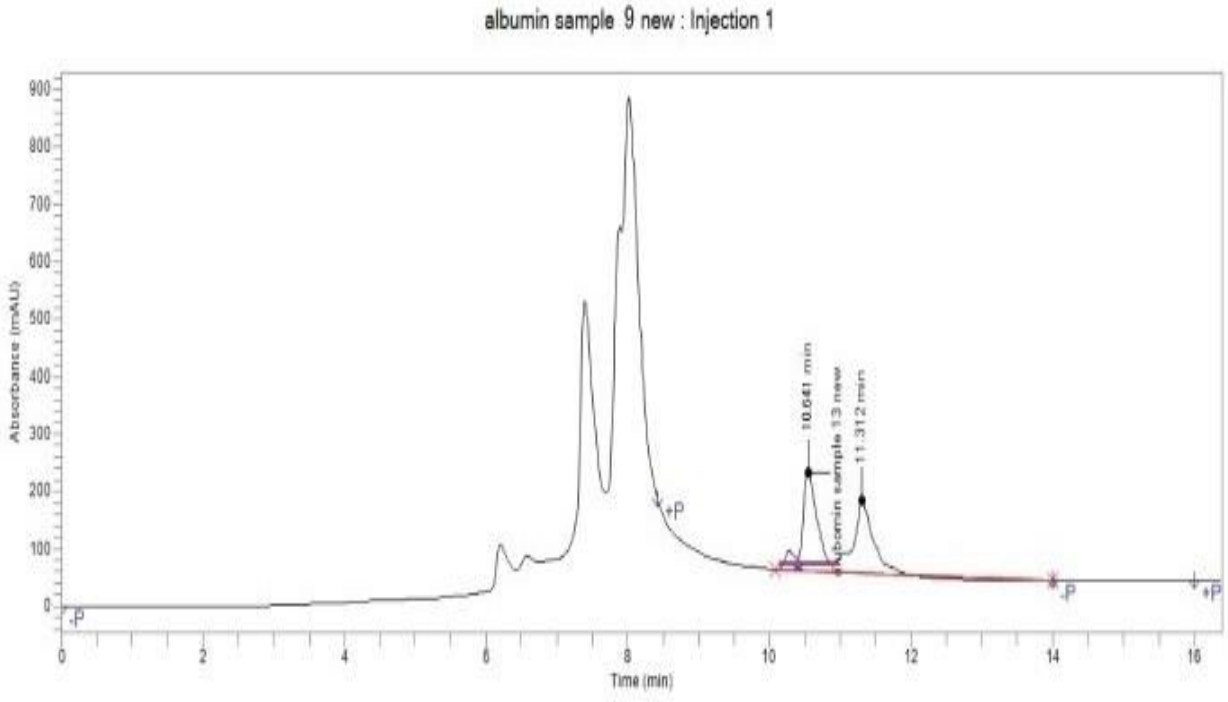

Fig. 4: chromatogram of the HSA-sample-B.

Table 5: The results obtained in the pharmaceutical preparation (A) from the study and permitted by the world health organization

Sample	Mean	SD	% RSD	Concentration of rHSA-sample in 50 g/ml	International pharmacopoeia
1	10.853	0.8	0.21	3.2	**\10g/50ml**
2	10.735	0.02	0.19	4.88	**10g/50ml**
3	10.990	0.006	0.05	0.46	**10g/50ml**
4	10.794	0.02	0.19	0.78	**10g/50ml**
5	10.854	0.02	0.18	3.5	**10g/50ml**
6	10.998	0.004	0.03	0.0095	**10g/50ml**

Table 6: The results obtained in the pharmaceutical preparation (B) from the study and permitted by the world health organization

Sample	Mean	SD	% RSD	The concentration of sample in 50ml	International pharmacopoeia
1	10.782	0.007	0.06	3.6	**10g/50ml**
2	10.700	0.04	0.06	2.6	**10g/50ml**
3	10.680	0.04	0.37	0.4	**10g/50ml**
4	10.885	0.006	0.05	1.5	**10g/50ml**
5	10.750	0.003	0.03	2.5	**10g/50ml**
6	10.750	0.02	0.16	4.1	**10g/50ml**

Conclusions

Based on the results obtained from this study, we conclude the following:

1- All the samples do not meet the specifications of the world health organization for medicines, and each sample should contain 10g per 50 ml.

2- The tail first peak of N-Acetyl-Tryptophan (4.85min) is distorted and divided, as well as the second peak of HAS (10.50min) tail split and distorted. This indicated that these preparations were exposed to the oxidation process during the smuggling process due to poor storage

Recommendations

Based on the results obtained, the study recommends the following:

1- Monitoring the pharmaceutical market through continuous inspection of drugstores and pharmacies.

2- The need to update and develop laws to tighten the penalties imposed on drug fraud offenses.

3- Activating the role of scientifically, technically and humanly equipped laboratories to detect harmful or adulterated ingredients, especially in pharmaceutical products.

4- Tightening control over pharmacies and closing pharmacies that buy and sell such medicines.

5- Alerting doctors of the dangers of counterfeit medicines and punishing those who prescribe these medicines.

6- Spreading awareness among community members of the health and economic harms of counterfeit medicines, and increasing the approved budget for health awareness.

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مقالة بحثية

تقييم جودة المستحضرات الصيدلانية لألبومين المصل البشري المتوفرة في الصيدليات بمحافظة عدن - اليمن

أحمد ثابت أحمد السرحي[1،*] و مطيع عيدروس سعيد[2]

[1] قسم الكيمياء، كلية العلوم، جامعة عدن، اليمن
[2] قسم الكيمياء، كلّيّة التربية - صبر، جامعة لحج، اليمن

* الباحث الممثّل: أحمد ثابت أحمد السرحي؛ البريد الالكتروني: ahmedbiochem@gmail.com

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المُلخّص

الألبومين له أهمية بيولوجية اذ يقوم بمجموعة من الوظائف في جسم الانسان مثل المحافظة على الضغط الأسموزي للسوائل و نقل الادوية والأيونات وغيرها، فان عملية غش مثل هذه المستحضرات الصيدلانية يودي الى تفاقم الحالة المرضية وقد يودي الى الوفاة احيانا. ففي هذه الرسالة تم دراسة نوعين من المستحضرات الصيدلانية للألبومين الغير مصرح لها من قبل وزارة الصحة اليمنية والتي دخلت البلاد عن طريق التهريب وهي اكثر مستحضرات الالبومين تواجدا في صيدليات ومستشفيات محافظة عدن – اليمن اثناء فترة الدراسة حيت رمز للمستحضر الاول بالرمز A والثاني بالرمز B. تم تحليل عينات الدراسة في مختبر الهيئة العامة للأدوية والمقاييس عدن-اليمن باستخدام جهازكروموتوجرافي السائلة عالية الاداء HPLC مثل الطور الثابت كولوم C4 (mm250 × mm4.6) المحتوي على مادة السيليكا المغلفة بالبيوتيل، والطور المتحرك يمثل مادة الاسيتونتريل مع حمض ثلاثي فلورو اسيتك بنسبة (1 – 99%). تم التحقق من مصداقية ودقة الطريق وحدود الكشف بتحضير سته محاليل قياسية من مادة الالبومين القياسيه في المدى (0.1 – 5g/ml) فكان حد الكشف (LOD) و(LOQ) يساوي 0.23، 0.72 على التوالي. وبعدها تم قياس ست عينات من كل مستحضر بنفس الطريقة. اثبتت نتائج الدراسة ان هذه المستحضرات لا تنطبق عليها مواصفات الجودة منظمة الصحة العالمية، بالإضافة لتعرضها لدرجات حرارة وسواء خزن ادى الى تاكسدها كانت نتائج تحليل جميع العينات اقل من الحد المسموح به، لأن كل عينة يجب أن تحتوي على 10 جرام لكل 50 مل.

الكلمات المفتاحية: الالبومين، الكروموتاجرافيا السائلة عالية الأداء، الصيدلانية، عدن.

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