ORIGINAL ARTICLE

A C G publications

J. Chem. Metrol. 17:1 (2023) 93-99

journal of chemical metrology

Determination of adulteration in hair serums by LC-HRMS §

T. Çağdaş Akaslan [1*], Şule Yalçın [2], Ayşenur Günaydın Akyıldız [3] and Ahmet C. Gören [4*]

[1]*Bezmialem Vakif University, Faculty of Medicine, Department of Skin and Venereal Diseases, Fatih, İstanbul, Türkiye*

[2]*Bezmialem Vakif University, Drug Application and Research Center (İLMER), Fatih, İstanbul, Türkiye*
[3]*Bezmialem Vakif University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Fatih, İstanbul, Türkiye*
[4]*Gebze Technical University, Faculty of Basic Sciences, Department of Chemistry, Gebze, Kocaeli, Türkiye*

(Received May 02, 2023; Revised June 22, 2023; Accepted June 25, 2023)

Abstract: Minoxidil, finasteride, dutasteride, and clobetasol propionate might be present as adulteration agents in cosmetic products such as hair serums. In this study, minoxidil, finasteride, dutasteride, and clobetasol propionate in commercial hair serum samples are determined by an LC-HRMS method. The relative standard deviations (R.S.D.) were below 1.5% for the compounds. The correlation coefficient was determined in the range of 0.997-0.999 for each component in the calibration range. The recoveries of the method are determined as 97.5, 105.5, 104.2, and 100.8, respectively. These active pharmaceutical ingredients were not declared by the manufacturer, however, pose risk to public health due to their potential toxicological properties. The manufacturers and government authorities must be aware and give importance to inform the users to prevent the possible risks.

Keywords: Minoxidil; clobetasol propionate; finasteride; dutasteride; hair serum; toxicology.

1. Introduction

Androgenic alopecia (AGA); i.e., male pattern baldness, a common condition characterized by thinning of scalp hair, has an important place as one of the important cosmetic problems today. The androgenic agent testosterone is converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase, and the metabolite plays a crucial role in AGA pathogenesis. In this regard, one of the current treatment methods is designed to prevent the formation of DHT. Finasteride and minoxidil are only two drugs which currently have FDA approval in AGA treatment. The first prevents the formation of DHT by blocking the 5α-reductase enzyme and the latter aims to strengthen vascularization and hair follicles by vasodilatation [1,2].

Minoxidil is a potent vasodilator which was first introduced as a treatment for hypertension [3]. It has strong hair growth inducing effects which is possibly linked to the activation of the β-catenin pathway [4]. Finasteride and dutasteride, medications that inhibit type II 5α-reductase, are also used for the treatment used for AGA [5]. Clobetasol propionate is a potent topical steroid which is used in the

* Corresponding author: E-Mail: akaslan.cagdas@gmail.com (T.C. Akaslan) acgoren@gtu.edu.tr (A.C. Goren)
§The study was presented in 11th TCS Cosmetic Chemistry Congress, 26-28 March 2021, Kemer- Antalya-Türkiye.

The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology January-June 2023 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.90.2304.2599
Available online: June 29, 2023

treatment of atopic and contact dermatitis, psoriasis, and vulvar lichen sclerosus [6]. Additionally, in hair serum applications special rollers are used to easily reach the bottom of the preparations applied to the roots of the follicles, and cracks are required to form in the skin. Since this causes damage to the skin, there is also the possibility that steroids such as clobetasol propionate have been used for prevention or healing.

Several studies have been reported for the measurement of the minoxidil [7], finasteride [8], dutasteride [9] and clobetasol propionate [10] in the literature to define and quantify them in their pharmaceutical dosage forms and pharmacokinetic experiments.

Therefore, in this study, it is aimed to develop a fast, precise, and reliable LC-HRMS method to investigate whether these active ingredients existence in commercially available three hair serums which claim to consist of all-natural ingredients. The possible toxic effects of these molecules on human health are evaluated herein.

2. Experimental

2.1. Materials

Minoxidil (≥99 % Sigma-Aldrich), Finasteride (≥98 % Sigma-Aldrich), Dutasteride (≥96 % Sigma-Aldrich), Clobetasol propionate (European Pharmacopoeia Reference Standard). Ammonium format, formic acid obtained from Merck. HPLC grade methanol and acetonitrile purchased from Sigma-Aldrich. HPLC-grade water produced on Elga Milli-Q water (Bedford, MA, UK) water purification system.

2.2. Standard Solutions

Standard solution mixtures were prepared at 6 different concentrations (0.1 mg/L, 1 mg/L, 10 mg/L, 25 mg/L 50 mg/L and 100 mg/L) in methanol. 1000 mg/L of stock solution of dihydrocapsaicin (purity 97%) in methanol was used as an internal standard.

2.3. Optimization of LC-MS Conditions

The optimum mobile phase conditions were determined for the target compounds, a gradient of methanol and water system. mobile phase A composed of 0.1 % ammonium formate in methanol and mobile phase B composed of 1% ammonium formate in water. The mobile phases for HPLC composed of the following gradients of mobile phases of (A) and B. 0-1.00 min 50% A and 50% B, 1.01-3.00 min 100% B, 3.01-6.0 min B %, and 6.01-15.0 min 50% A and 50% B. (flow rate, 0.35 mL/min; column temperature, 22 ° C). Environmental conditions were adjusted 22.0 ± 5.0 °C and a relative humidity of 50 % ± 15 %. A Thermo Scientific ORBITRAP Q-EXACTIVE mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a Troyasil C18 column (150 x 4.6 mm i.d., 5 mm particle size). Electro-spray ionization (ESI) ionization technique was used for the ionization. Ions between *m/z* 100 and 900 were scanned in high-resolution mode for the target compounds. The MS conditions were used as following: sheath gas flow rate 45, auxiliary gas flow rate, 10, spray voltage, 3.80 kV, capillary temperature, 320 °C, auxiliary gas heater temperature 320 °C, S-lens RF level 50. Compounds were identified by comparing the retention times of standards. Dihydrocapsaicin (purity 97%) was used as an internal standard. Table 2 shows the ionization mode, mass data, linear range, linearity, limit of detection (LOD), limit of quantification (LOQ) and recovery information [11-14].

2.4. Method Validation

An LC-HRMS method was developed for determination of minoxidil, finasteride, dutasteride and clobetasol propionate in commercial hair serums. Method validation parameters were determined as specificity, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ) and repeatability (in a day and intra days).

2.4.2. Specificity

The specificity is an important tool as a measure of how accurately and precisely the target analyte can be identified in the matrix without any interference. The specificity of the developed LC-HRMS method was determined by direct analysis (blank) of the prepared methanol extract of *Serenoa serrulata* and spiked target analytes [15-17].

2.4.3. Accuracy

Accuracy is the closeness of agreement between the value of the amount of substance of the measurand in the matrix and the nominal quantity value of the measurand in it. In this study, accuracy was determined for each compound at three different concentrations, 1 mg/L, 25 mg/L, and 50 mg/L. The values obtained by measuring the samples prepared gravimetrically at these concentrations were calculated using the following recovery formula and the results are given in Table 1 [15-17].

%Recovery = Recovered concentration /Injected concentration x 100

2.4.4. Precision

Three different concentrations (1 mg/L, 25 mg/L, and 50 mg/L) were used in intra-day and inter-day precision studies. For the precision study, all concentration levels were analyzed 6 times on 3 different days. The relative standard deviation (RSD) was calculated using the formula below. Acceptance limits for % RSD of the measurands were determined as less than 1.5 %.

%Relative standard deviation = Standard deviation/Mean x 100

2.4.5. Detection and Quantitation Limits

LOD and LOQ studies were carried out by spiking the starting material at lower mass fraction values such as 0.05 mg/L and 0.1 mg/L of the compounds. The developed LC-HRMS method was applied to the 6 spiked samples. The LOD and LOQ values were calculated by multiplying the standard deviation (SD) by 3 and 10, respectively (see Table 1) [17].

2.4.6. Linearity

The linearity of the LC-HRMS method was assayed by analyzing standard solutions in the range of 0.1-100 mg/L for minoxidil, finasteride, dutasteride and clobetasol propionate. The R^2 values were found to be in the range of 0.997-0.999. The linear regression equations were reported in Table1.

2.5. Sample Preparation

Approximately 10 mL of hair serum sample was filtered on a 0.45 µm nylon filter. Then, 4 mL of filtrate sample was placed in a 5 mL volumetric flask and 50 µL of internal standard stock solution was added in it. Then it was adjusted to the volume with filtered sample solution. From the final solution 1.5 mL was taken and transferred to the HPLC vial and, from the vial 2 µL was injected to the LC-HRMS.

2.6. Measurement Uncertainty Assessment

The uncertainty parameters were determined as purity of the standards, weighing of sample intake, repeatability, recovery, and calibration curve. The EURACHEM CITAC methodology was used for the estimation of the measurement uncertainty. Since the detailed methodology and used equation were given below and in our previous reports in detail (see Table 2) [15-17].

$$U_{combined} = \sqrt{u_{s\,purity)}{}^2 + (u_{weighing)}{}^2 + (u_{calibration)}{}^2 + (u_{recovery)}{}^2 + (u_{repeatability)}{}^2}$$

3. Results and Discussion

3.1. Analytical Chemistry

In this study, a simple and widely applicable an analytical method for determination of minoxidil, finasteride, dutasteride, and clobetasol propionate was developed by using LC-HRMS technique in hair serums which are used for the treatment of AGA. The developed method was validated according to the EURACHEM CITAC Guide [17]. Method validation parameters are summarized in Table 1. The uncertainty budget of each compound is given in Table 2. Since the uncertainty value from the weighing parameter is very small, it has been neglected in the calculation. The main sources of the uncertainty are determined as repeatability, calibration curve, and purity of the compounds.

Samples produced in different batches of the three best-selling hair serum samples in Türkiye were obtained from the market and analyzed according to the developed method. As a result of the analyzes made, the presence of high amounts of synthetic drugs/actives were detected in the hair serum samples declared by the manufacturer to be of herbal origin. When Figure 1 is examined, it has been determined that the hair serum sample with F code contains minoxidil, finasteride, and clobetasol propionate, while the presence of minoxidil, finasteride, and dutasteride was determined in the serum sample with the S code. Lastly, in the T coded serum, these compounds have not been detected.

Table 1. Method validation parameters of the developed method

Compound	*m/z*	Ionization Mode	Linear Range	Linear Regression Equation	LOD/ LOQ	R^2	Recovery
Minoxidil	210.1347	Positive	0.1-100	y=0.1194x+0.7918	0.03/0.09	0.999	97.5
Finasteride	373.2846	Positive	0.1-100	y=0.149x+0.3777	0.03/0.09	0.998	105.2
Clobetasol propionate	467.1971	Positive	0.1-100	y=0.0001357x-0.0000786	0.03/0.09	0.999	100.8
Dutasteride	529.2285	Positive	0.1-100	y=0.08042x+0.3711	0.03/0.09	0.997	104.2

* Linear regression analysis with a calibration equation of y = ax + b in which x is the concentration in µg/mL the compound and y is the peak area.

Table 2. Determined Active substances in commercial hair serum samples (mg/L)

Compound	Hair Serum F	Hair Serum S	Hair Serum T	U %
Minoxidil	25612.44	29625.35	<LOD	2.46
Finasteride	6150.55	3125.7	<LOD	3.53
Clobetasol propionate	2202.85	<LOD	<LOD	3.72
Dutasteride	<LOD	113.01	<LOD	4.47

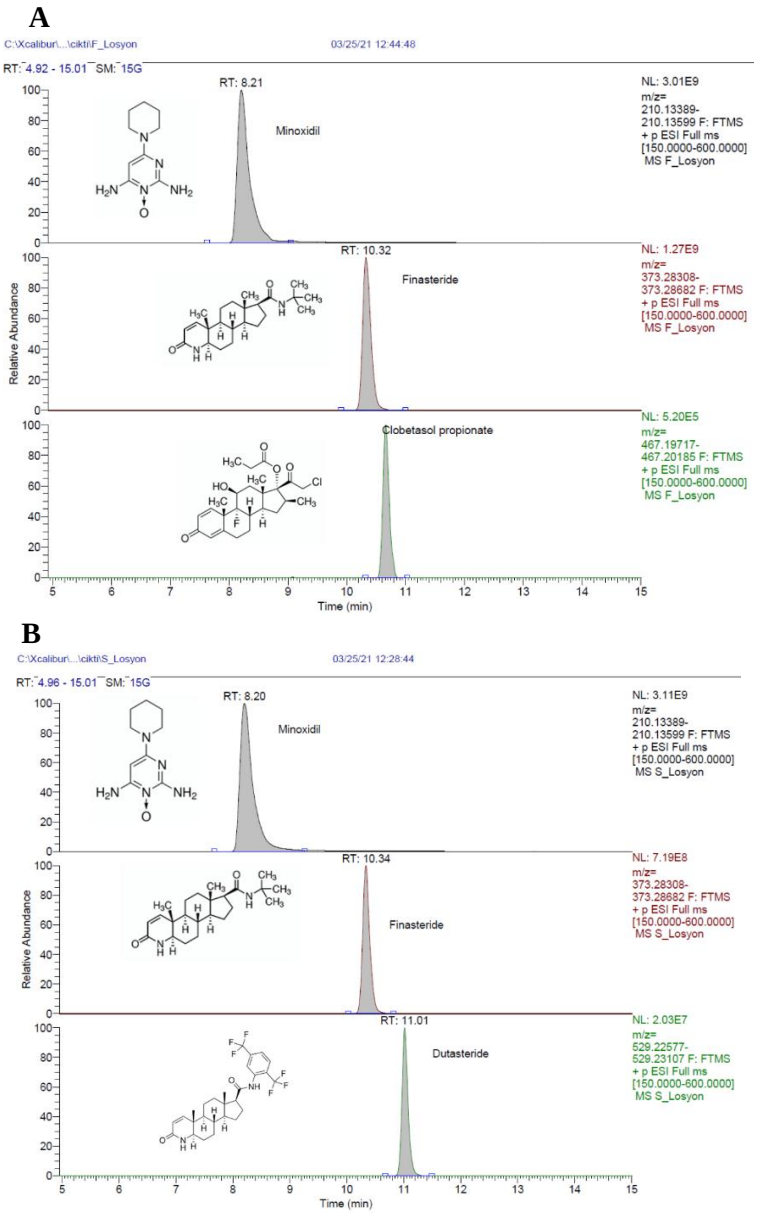

Figure 1. LC-HRMS chromatograms of commercial hair serum samples **A)** Commercial hair sample coded F, **B)** Commercial hair sample coded as S

3.2. Potential Toxic Effects of the Ingredients

The active ingredients in any cosmetic product in Türkiye must be declared to the Ministry of health, Turkish Medicines and Medical Devices Agency. However, the presence of the compounds detected here has not been declared by the manufacturer. Considering that finasteride is not licensed in liquid form in Türkiye, this study highlights the importance of reporting and regulation. Moreover, the possible toxic effects of these compounds pose serious risks to public health.

In this study, minoxidil, finasteride, and dutasteride were determined in two different hair serum (lotion) brands it the Turkish market. In the hair serum F and S the major active ingredient was found to be as minoxidil, 25612.44 mg/L and 29625.35 mg/L, respectively. In hair serum F, finasteride and Clobetasol propionate were determined, while in the hair serum S, finasteride and dutasteride were determined as an adulteration agent. None of these compounds are declared on the labels of the examined

products. In this regard, the toxicological properties of the determinant compounds are of great importance.

Minoxidil is a potent vasodilator, when applied topically it may cause undesirable hair texture and irritant contact dermatitis, therefore, it can be preferred to be used orally [18]. However, in case of oral exposure, it may cause undesired systemic effects such as cardiovascular toxicity [19] besides hypertrichosis which is excessive hair growth anywhere on the body [3].

The next determined compounds in the serums are finasteride and dutasteride. They may cause systemic toxic effects such as erectile dysfunction, ejaculation problems, decreased libido which is linked with its antiandrogenic effects. Additionally, an increased risk of depression is an important adverse effect of them [5,20]. Although topical administration is well tolerated, excessive topical administration might lead to these systemic toxic effects.

The other determined compound in the studied hair serums is clobetasol propionate. Its mechanistic effects are related to its anti-inflammatory, immunosuppressive and antimitotic effects, which is linked with growth and differentiation inhibiting cytokine production. Indeed, it has local and systemic side effects, such as skin atrophy and hypothalamic-pituitary-adrenal axis suppression [6]. We claim that clobetasol propionate has been placed in this serum mixture to reduce possible irritation which may be caused due to uncontrolled usage of active ingredients. Besides that, in hair serum applications, special rollers are used to easily reach the bottom of the preparations applied to the follicles, and cracks are required to form in the skin. Since this has caused damage to the skin, there is also the possibility that steroids such as clobetasol propionate have been used to prevent this or accelerate healing.

4. Conclusion

In conclusion, a simple and effective LC-HRMS method for minoxidil, finasteride, dutasteride, and clobetasol propionate in hair serums was developed and validated in this study. This developed method was recorded in the literature with this study as the first method performed simultaneously for these four metabolites. In the hair serum samples sold in the market in Türkiye, active pharmaceutical ingredients that were not declared by the manufacturer were detected. This situation poses a danger both in terms of competition among companies and public health. It is very important to use those drugs under the control of a physician and to monitor them continuously to prevent possible symptoms. Hereby, minoxidil, finasteride, dutasteride, and clobetasol propionate might be present is hair serums as adulteration agents. The manufacturers and government authorities must be aware and give importance to inform the users to prevent the possible risks for public health.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

ORCID
T. Çağdaş Akaslan: 0000-0002-7349-5783
Sule Yalcin: 0000-0003-4974-2905
Ayşenur Günaydın Akyıldız: 0000-0003-1196-9530
Ahmet C Gören: 0000-0002-5470-130X

References

[1] J.H. Kalin and J.A. Bergman (2013). Development and therapeutic implications of selective histone deacetylase 6 inhibitors, *J. Med. Chem.* **56**, 6297-6313.

[2] P. Molyneux (1984). Water-soluble Synthetic Polymers: Properties and Behavior, 2'd Ed., CRC Press, Boca Raton, Florida

[3] P. Suchonwanit, S. Thammarucha and K. Leerunyakul (2019). Minoxidil and its use in hair disorders: a

review, *Drug Design. Develop. Therap*. 13, 2777-2786. doi: 10.2147/DDDT.S214907.

[4] M.H. Kwack, B.M. Kang, M.K. Kim, J.C. Kim and Y.K. Sung (2011). Minoxidil activates β-catenin pathway in human dermal papilla cells: A possible explanation for its anagen prolongation effect, *J. Dermatol. Sci.* **62**(**3**), 154-159.

[5] B.M. Piraccini, U. Blume-Peytavi, F. Scarci, J.M. Jansat, M. Falqués, R. Otero, M. L. Tamarit, J. Galván V. Tebbs and E. Massana andTopical Finasteride Study Group (2022). Efficacy and safety of topical finasteride spray solution for male androgenetic alopecia: A phase III, randomized, controlled clinical trial, *J. Eur. Acad. Dermatol. Venereol.* **36**(**2**), 286-294.

[6] R. Pels, W. Sterry and J. Lademann (2008). Clobetasol propionate--where, when, why?, *Drugs Today* **44**(**7**), 547-57.

[7] P.M. Oliveira, T. Sampaio, L.C.F. Franca, T. Grateri, M. Cunha and G.M. Gelfuso (2020). LC-MS bioanalytical method for simultaneous determination of latanoprost and minoxidil in the skin, *J. Pharmaceut. Bimed. Anal.* **187**, 113373. doi.org/10.1016/j.jpba.2020.113373.

[8] A.K. Kammoun, M.T. Khayat, A.J. Almalki and R.M. Youssef (2023).Development of validated methods for the simultaneous quantification of Finasteride and Tadalafil in newly launched FDA-approved therapeutic combination: greenness assessment using AGP, analytical eco-scale, and GAPI tools, *RSC Adv.* **13**(**17**), 11817-11825.

[9] K.A.M.Attia, A. Serag, S. Eid, A.E.F. Abbas (2022). A new chemometrically assisted uv spectrophotometric method for simultaneous determination of tamsulosin and dutasteride in their pharmaceutical mixture, *JAOAC Int.***105**(**6**), 1755-1761..

[10] P.P. Yang, W. Huang, L.X. Li and H. Liu (2023). Determination of new glucocorticoid called clobetasol acetate in cosmetics by ultra performance liquid chromatography-tandem mass spectrometry, *Chinese J. Chromatogr.* **41** (**3**), 250-256

[11] H. Kiziltas, A.C. Goren, Z. Bingöl, S.H. Alwasel and I. Gulcin (2021). Anticholinergic, antidiabetic and antioxidant activities of *Ferula orientalis* L. determination of its polyphenol contents by LC-HRMS, *Rec. Nat. Prod.* **15**, 513-528.

[12] A.C. Gören, G. Bilsel and M. Bilsel. (2007). Rapid and simultaneous determination of 25-OH-vitamin D 2 and D 3 in human serum by LC/MS/MS: Validation and uncertainty assessment, *J. Chem. Metrol*. **1**, 1-9.

[13] N. Boke Sarikahya, G.S. Okkali, D. Gunay, A.C. Goren, F.O. Coven and A. Nalbantsoy (2022). LC-HRMS based approach for identification and quantification analysis of chemical constituents of sea cucumbers from aegean sea-their cytotoxic and antiviral potentials, *Rec. Nat. Prod.* **16**, 592-604.

[14] N. Boke Sarikahya, A.C. Goren, G.S. Okkali and S. Kirmizigul (2021). Saponins from twenty-two cephalaria species, *Rec. Nat. Prod.* **15**, 537-546.

[15] H. Kızıltaş, Z. Bingöl, A.C. Gören, S.M. Pinar, S.H. Alwasel and İ. Gülçin (2021). LC-HRMS profiling of phytochemicals, antidiabetic, anticholinergic and antioxidant activities of evaporated ethanol extract of *Astragalus brachycalyx* Fischer, *J. Chem. Metrol*. **15**, 135-151.

[16] A.Kul (2022). Simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate and paracetamol by HPLC-PDA in pharmaceutical dosage forms, *J. Chem. Metrol*. **16**, 102-110.

[17] B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The fitness for purpose of analytical methods – a laboratory
guide to method validation and related topics, (2nd ed. 2014). ISBN 978-91-87461-59-0

[18] M. Randolph and A. Tosti (2021). Oral minoxidil treatment for hair loss: a review of efficacy and safety. *J. Am. Acad. Dermatol.* **84**(**3**), 737-746.

[19] G. Hanton, C. Sobry, N. Daguès, G.Y. Rochefort, P. Bonnet and V. Eder (2008). Cardiovascular toxicity of minoxidil in the marmoset. *Toxicol. Lett.* **180**(**3**), 157-165.

[20] T. Arif, K. Dorjay, M. Adil and M. Sami (2017). Dutasteride in androgenetic alopecia: an update. *Curr. Clin. Pharmacol.* **12**(**1**), 31-35.