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***Dedicated to Professor Emil Cordoș on the occasion of his 80$^{th}$ anniversary***

# DETERMINATION OF ANDROSTERONE FROM CELERY BY A NEW VALIDATED LC-MS/MS METHOD

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**ABSTRACT.** Celery is recently the subject of various studies due to its role in human nutrition and for medicine purposes. One of the most speculated ideas is that celery contains high quantities of androsterone which makes it suitable for infertility treatments. Due to this trend, the purpose of this study was to develop an analytical method suitable to confirm and measure the quantity of androsterone from celery root. A LC-MS/MS method was developed using a Turbo V source in positive ionization mode. Analytical parameters such as: linearity, detection and quantification limits, accuracy and precision and matrix effect were evaluated. The calibration curve was developed in the range of 100 to 400 ng/ml with a correlation coefficient $r^2$ of 0.9968 and detection limit of 10 ng/ml. The extraction method was tested for the recovery degree. The recovery obtained was 92.1±2.2%. The method was used to determine the content of androsterone from three celery varieties from Romanian market.

***Keywords:*** *androsterone, celery, solid-liquid extraction, detection limits, accuracy.*

## INTRODUCTION

During ancient times, celery (*Apium graveolens, Apiaceae family*) was used only for medical purposes such as: treatments for colds, flu, water retention, poor digestion, different types of arthritis, and certain diseases of

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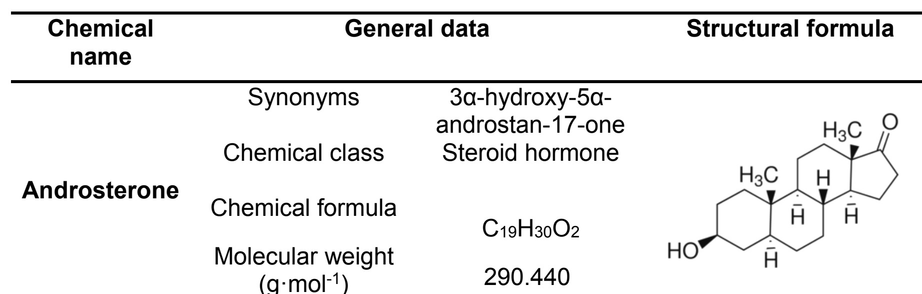
the liver and spleen [1]. Celery begins to be used as a vegetable in Italy in the fourth century [2] and Britain by sixteenth century [3]. Nowadays, all parts of celery are used for both nutrition and medicine purposes.

It was proven so far that celery contains compounds such as: phalides, coumarrins [4], fatty acids [5], folate, potassium, molybdenum, small amounts of vitamin C, vitamin A and some B vitamins, flavonols and flavone antioxidants, androsterone [6, 7] and androstenol [6], which have many health benefits. Some of these benefits are: anti-rheumatic, hypoglycemic, sedative, antiseptic for urinary tract, blood pressure lowering, diuretic, analgesic, anti-inflammatory, detoxification, anti-spasmodic, anti-bacteria and stomach tonic [4-6]. A special attention is given to the potential of celery to have aphrodisiac effects [7] especially nowadays, when infertility is a major health problem considered to be directly related to other health problem such as coronary heart diseases and diabetes or caused by exposure to different toxic factors, chronic smoking, alcohol intake and prolonged exposure to contaminants and air pollutants [7]. Until now, few studies are available on this subject [8-10]. They tried to quantify the androsterone content by chromatographic means such as: GC-MS [8, 9] and LC-MS/MS techniques [8, 10].

The present study aims to develop an easy and reliable method for determining the androsterone (Table 1) content in celery root by means of LC-MS/MS.

**Table 1.** Basic information about Androsterone [11]



| Chemical name | General data | | Structural formula |
|---|---|---|---|
| Androsterone | Synonyms | 3α-hydroxy-5α-androstan-17-one | |
| | Chemical class | Steroid hormone | |
| | Chemical formula | $C_{19}H_{30}O_2$ | |
| | Molecular weight ($g \cdot mol^{-1}$) | 290.440 | |

## RESULTS AND DISCUSSION

### LC-MS/MS profile

Several experiments were performed in order to establish the MS/MS parameters: the ionization mode, the precursor and product ions (MRM transition), the parameters specific to the MS/MS method and HPLC parameters. These parameters are presented in Table 2.

The MRM (multiple reaction monitoring) transition of the pair 291.4→273.4 for 400 ng/ml of Androsterone is presented in Figure 1.

**Table 2.** LC-MS/MS parameters for determining Androsterone

| **Compound** | **Androsterone** |
|---|---|
| **MS/MS parameters** | |
| Ionization mode | Pozitive |
| Compound dependent parameters | DP (V):51.00; EP (V):5.50; CEP (V): 12.00; CE (V): 13.00; CXP (V):4.00 |
| Source dependent parameters | CUR:10.00 psi; CAD:Medium; IS:5500.00V; TEM:450°C; GS1:35.00 psi; GS2:20.00 psi |
| MRM transition | 291.4→273.4 |
| **HPLC parameters** | |
| Chromatographic column | Phenomenex Synergi Fusion 2.5µm, 2×50 mm |
| Flow rate | 0.5 ml/min |
| Column temperature | 20°C; |
| Injection volume | 40 µl |
| Mobile phase: | A:B (90:10 v/v); where A: $CH_3CN$+ 0.1% $HCO_2H$ and B: $H_2O$ + 0.1% $HCO_2H$ |

### Method evaluation

In order to validate the developed LC/ESI(+)-MS/MS method the following parameters were evaluated: retention time, linear range, linear equation, correlation coefficient, detection and quantification limits and accuracy. The obtained values are presented in Tables 3 and 4. The matrix effect was 6% and was not taken in consideration for further investigations. The recovery of the extraction method was evaluated and obtained result was 92.1±2.2%.

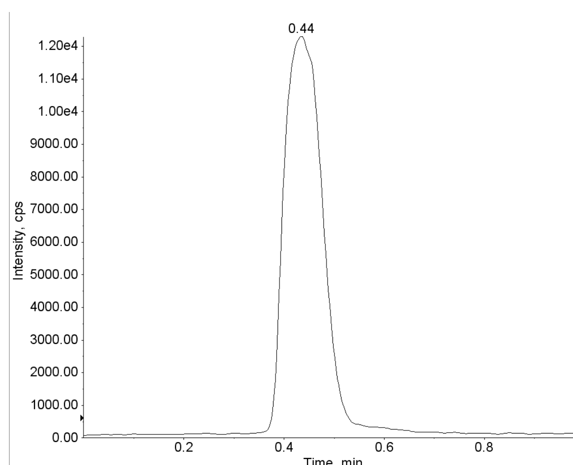



**Figure 1.** MRM chromatogram obtained for 400 ng/ml of Androsterone

**Table 3.** Experimental parameters of LC-ESI-MS/MS method for L-α-phosphatidylcholine determination

| Analyte | L-α-phosphatidylcholine |
|---|---|
| **Retention time $t_R$ (min)** | 0.44 |
| **Linear range (ng/ml)** | 100-400 |
| **Linear equation** | $1.44x+1.38*10^3$ |
| **Correlation coefficient $r^2$** | 0.9968 |
| **Detection limit LOD (ng/ml)** | 10 |
| **Quantification limit LOQ (ng/ml)** | 30 |

**Table 4.** Statistical parameters of LC-MS/MS method for Androsterone determination

| Statistical parameters | Concentration (ng/ml) | | |
|---|---|---|---|
| | 140 | 240 | 340 |
| **Intra-day** | | | |
| **Mean ±SD (ng/ml)** | 135.01±0.22 | 238.04±0.14 | 337.12±0.14 |
| **RSD (%)** | 0.16 | 0.06 | 0.04 |
| **Inter-day** | | | |
| **Mean ±SD (ng/ml)** | 132.22±0.26 | 235.88±0.28 | 335.12±0.41 |
| **RSD (%)** | 0.19 | 0.12 | 0.12 |

## Real sample experiments

Three varieties of celery root from Romanian market were tested for the androsterone content using the extraction and the LC-MS/MS methods described. The results varied from 7.44 – 12.03 mg/kg in wet celery. These results offer a first view on the Romanian celery market when referring to androsterone.

**Table 5.** The values obtained for androsterone in the investigated celery varieties

| Celery variety | Androsterone (mg/kg) | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| **Experimental values** | 7.44 | 12.05 | 9.81 |
| | 7.61 | 12.18 | 9.74 |
| | 7.72 | 12.31 | 9.61 |
| **MEAN** | **7.61** | **12.18** | **9.74** |

A rough analysis of the results shows that Androsterone represents between 0.0007% and 0.0012% from total celery which can be a considered a very low quantity but also one must have in mind that the percent of water in celery is very high.

## CONCLUSIONS

A LC-MS/MS method was developed for identification and quantification of Androsterone from celery. An HPLC Agilent 1200 series coupled with an ABI Sciex 3200 QTRAP mass spectrometer with a TurboV ionization source was used in ESI positive ion mode. The chromatographic column Phenomenex Synergi Fusion 2.5µm, 2×50 mm showed a good chromatographic peak. The specific parameters for mass spectrometer and also HPLC were identify and selected to assure the most sensitive response of the equipment. The calibration curve was developed in the range of 100 to 400 ng/ml with a correlation coefficient $r^2$ of 0.9968 and detection limit of 10 ng/ml. An extraction procedure was tested and the recovery was of value 92.1±2.2%.

The developed method was used to test the content of androsterone from three varieties of celery acquired from a Romanian market. The results show that the investigated celery varieties have very low content of androsterone.

## EXPERIMENTAL SECTION

### Standards and reagents

Androsterone was purchased from Dr. Ehrenstorfer. Methanol LC-MS Optigrade (≥99.8%), acetonitrile LC-MS Optigrade (≥99.8%) and formic acid (≥99.8%) were purchased from LGC Standards. Ultra pure water was obtained by using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA).

### Standard solution preparation

The stock solution (1mg/ml) was obtained by dissolving 1 mg of androsterone in 1 mL of $CH_3OH$. Six concentration levels of 100, 150, 200, 250, 350 and 400 ng/ml were prepared by diluting the stock solution with a mixture of $CH_3CN$/ $H_2O$ (90:10 v/v). These standard solutions were used for obtaining the calibration curve. Three concentration levels of 140, 240 and 340 ng/ml were prepared by diluting the stock solution with a mixture of $H_2O/CH_3CN$ (10:90 v/v) and were used for accuracy and precision studies.

### Sample extraction

The celery root was chopped and slowly dried in an oven at 35°C. 6mL of $(CH3)_2CO:CH_3OH$ (50:50, v/v) were added on 3 mg of chopped dried celery. The mixture was centrifuge for 10 min at 22°C with a speed of 4000 RPM. The supernatant was dried with a rotary evaporator and the content was reconstituted with $CH_3CN:H_2O$ (10:90 v/v).

### Analytical equipment

A high performance liquid chromatograph HPLC Agilent 1200 Series coupled with an ABI Sciex 3200 QTRAP mass spectrometer was used for this study.

### LC-MS/MS profile development

The development of LC-MS/MS profile has to follow several basic steps: identifying the ionization mode, establishing the compound dependent parameters (DP (declustering potential), EP (entrance potential), CE (collision energy) and CXP (collision cell exit potential)), establishing the source dependent parameters (CUR (curtain gas), CAD (collision gas), IS (ionspray voltage), TEM (temperature), GS1 (gas 1) and GS2 (gas 2)), choosing the optimal MRM (multiple reaction monitoring) transition, identifying the appropriate chromatographic column (the dimension of the particles, its length and diameter) and establishing the HPLC parameters. Only C18 columns were tested.

All these parameters have important roles in obtaining a strong and sensitive analytical method.

### Method validation

In order to validate the developed LC/ESI(+)-MS/MS the following parameters were evaluated: retention time, linear range, linear equation, correlation coefficient, detection and quantification limits, accuracy and precision.

*Linearity.* Six levels of concentration ranging from 100 to 400ng/ml were prepared by successive dilution with mobile phase from the stock solution. The calibration curve was obtained by plotting the peak area to corresponding concentrations. Useful information such as: linear equation and correlation coefficient were obtained.

*Detection and quantification limits*. Limit of detection (LOD) and limit of quantification (LOQ) were estimated by analyzing standard solutions at levels producing signals at signal-to-noise ratios of 3 and 10 respectively.

*Accuracy.* The intra- and inter- day accuracies by preparing three concentration levels which were used in both experiments. For intra-day study three replicas of three concentration levels were analyzed. For inter-day study three concentration levels were analyzed once per day for three consecutive days.

*Matrix effect.* The evaluation of matrix effect is very important in chromatography because it can cause signal suppression. In this study the target for matrix effect was proposed to be between -20% and +20. The value obtained for the analyte of interest after extraction was compared with pure solutions prepared in mobile phase containing equivalent amounts of the analyte of interest. The difference in response between the extracted sample and the pure solution multiplied by 100 and divided by the pure solution response determines the degree of matrix effect occurring to the analyte in question under chromatographic conditions [11]. For this method, the value obtain was 6%.

*Recovery.* The recovery (R) of the extraction method was determined by using the standard addition method. A sample of celery was extracted and then measured (*initial amount*). Then, other sample of the same celery was spiked with a known concentration of analyte (*spiked amount*), extracted and then measured (*final amount*).

The recovery was calculated using the following equation:

*R (%)=100×(final amount - initial amount)/spiked amount.*

## ACKNOWLEDGMENTS

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## REFERENCES

[1]. http://umm.edu/health/medical/altmed/herb/celery-seed;
[2]. A. Kokotkiewicz, M. Luczkiewicz, “Essential Oils in Food Preservation, Flavor and Safety, Flavor and Safety”, Elsevier B.V., London, **2016**, chapter 37.
[3]. G. Tobyn, A. Denham, M. Whitelegg, “The Western Herbal Tradition: 2000 years of medicinal plant knowledge”, Elsevier B.V., Edinburgh, **2011**, chapter 9.
[4]. M. Modaresi, G. Ghalamkari, A. Jalalizand, *APCBEE Procedia*, **2012**, *4*, 99.
[5]. H. B. Sowbhagya**,** *Critical Reviews in Food Science and Nutrition*, **2014**, *54(3),* 389
[6]. http://www.livescience.com/50640-celery-nutrition.html
[7]. M. A. Shalaby, H. Y. El Zorba, *Global Veterinaria*, **2010**, *5(2),* 122.
[8]. N. Krone, B. A. Hughes, G. G. Lavery, P. M. Stewart, W. Arlt, C. H.L. Shackleton, *The Journal of Steroid Biochemistry and Molecular Biology*, **2010**, *121(3-5)*, 496.
[9]. T. Trinh, N. B. Harden, H. M. Coleman, S. J. Khan, *Journal of Chromatography A*, **2011**, *1218*, 1668.

[10]. Y. C. Ma, H. Y. Kim, *Journal of the American Society for Mass Spectrometry,* **1997**, *8(9)*, 1010.
[11]. https://en.wikipedia.org/wiki/Androsterone
[12]. P. J. Taylor, *Clinical Biochemistry*, **2005**, *38*, 328.