5th International Electronic Conference on Medicinal Chemistry

1-30 November 2019

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Pharmacokinetics of Gestobutanoil in rat serum using HPLC-APCI-MS

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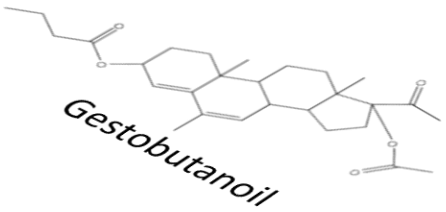
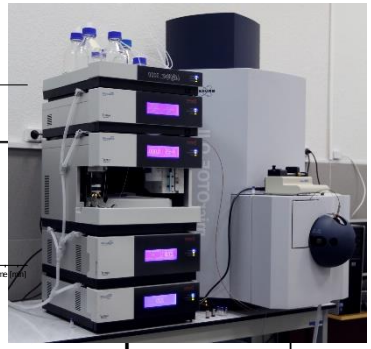
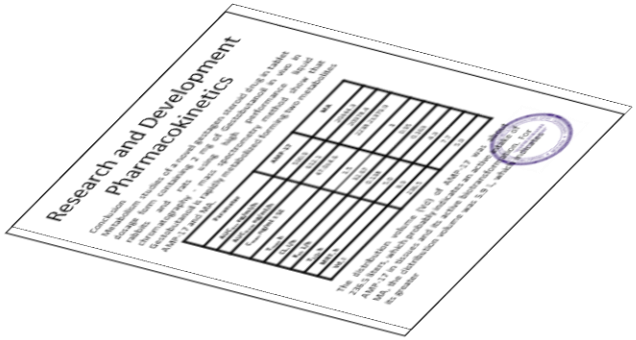






Abstract:

Pilot studies of the pharmacokinetics of Gestobutanoil (GB) showed that it undergoes rapid biotransformation forming two metabolites, which quantitative analysis was significant. An HPLC-MS technique was developed for the simultaneous quantification of GB and its two metabolites in rat serum. Due to the nature of GB and one of its metabolites, the ESI-MS-detection was ineffective. The ionization problem was solved with APCI. The detection of GB and one of its metabolites was carried out by the fragments of their molecules formed in the ionization chamber. The second metabolite formed [M+H]+ ions. During the method validation following characteristics were checked out: specificity, linearity, Precision, Accuracy, matrix effect, stability. The limit of quantification for each analyte was 10 ng/ml. Pharmacokinetics studies have shown that biotransformation of GB is so fast that it wasn't detected in rat serum even in 15 minutes after administration. The pharmacokinetics of two metabolites of GB was described.

Key words: Gestobutanoil; pharmacokinetics; HPLC-MS.

Introduction

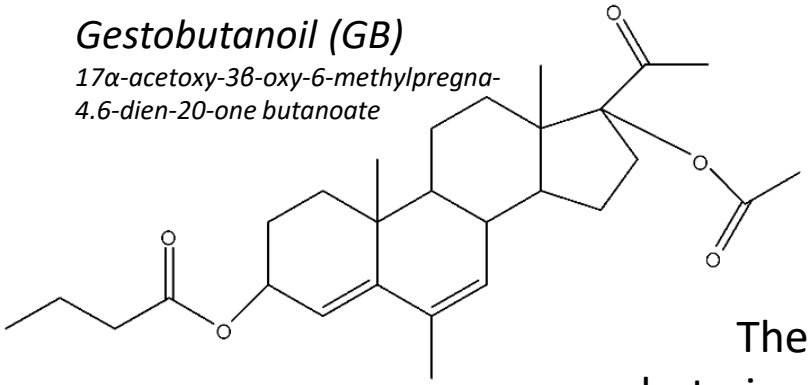
Progestins are widely used in the clinical practice of obstetrics, gynecology and endocrinology. Gestobutanoil is a new synthetic analogue of progesterone in tablet dosage form was developed in N.I.Pirogov Russian Medical Research University. The aim of this work was to investigate the bioavailability of Gestobutanoil and its two metabolites because their amount it rats blood was significant.

The quantification of three molecules in rat serum was performed using HPLC-MS. Molecules of steroid hormones and their synthetic analogues are of low polarity, which complicates their ionization through electrospray (ESI). Atmospheric pressure chemical ionization (APCI) is considered a harder method of ionization, but suitable for ionizing low-polar molecules.

Results and discussion

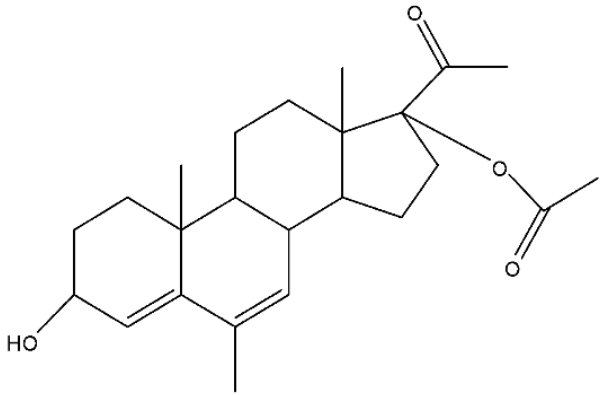
Gestobutanoil (GB)

17α-acetoxy-3β-oxy-6-methylpregna-4.6-dien-20-one butanoate



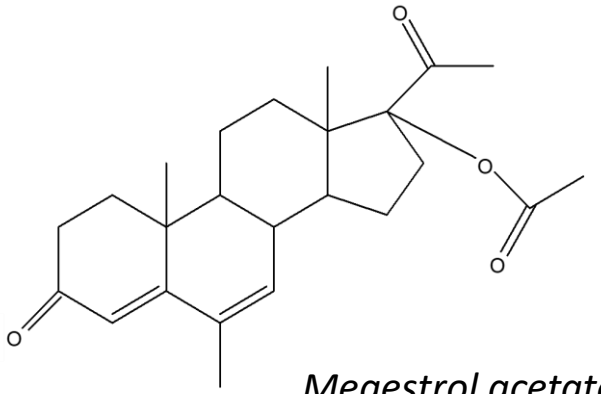
Pilot studies: rats were administered suspension of Gestobutanoil tablets (D=100 mg/kg). In this experiment GB wasn't detected in rat plasma. Having carefully studied the mass spectra of rat serum chromatograms. significant concentrations of two GB metabolites were revealed.

The metabolites were formed due to cleavage of butyric acid from the 3β position of the steroid core of GB.



Mepregenol acetate (AMP-17)

17α-acetoxy-3β-oxy-6-methylpregna-4.6-dien-20-one



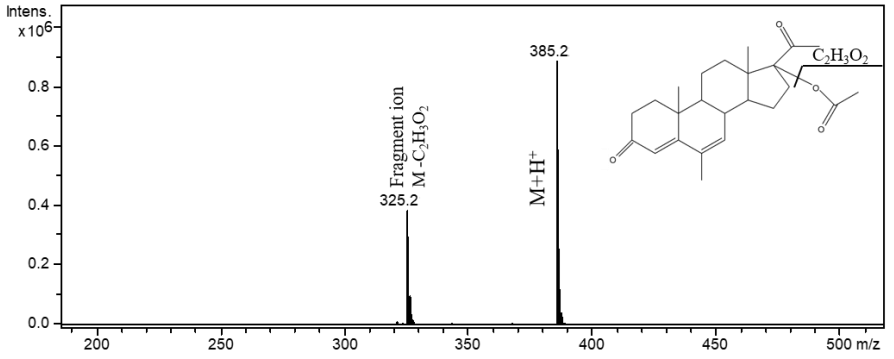
Megestrol acetate (MA)

17α-acetoxy-6-methylpregna-4.6-diene-3.20-dione

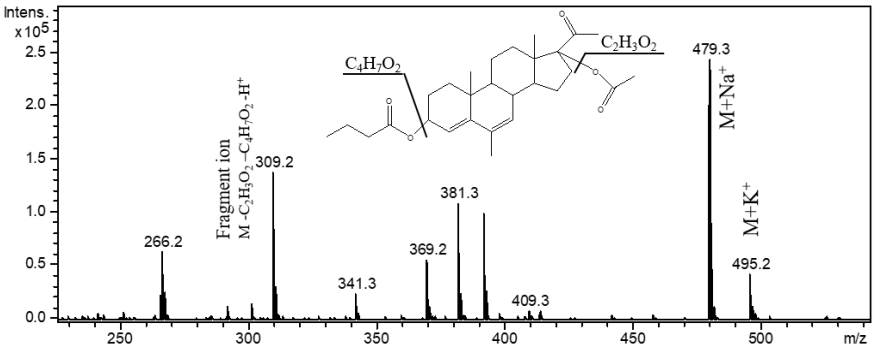
Results and discussion

ESI-MS spectra

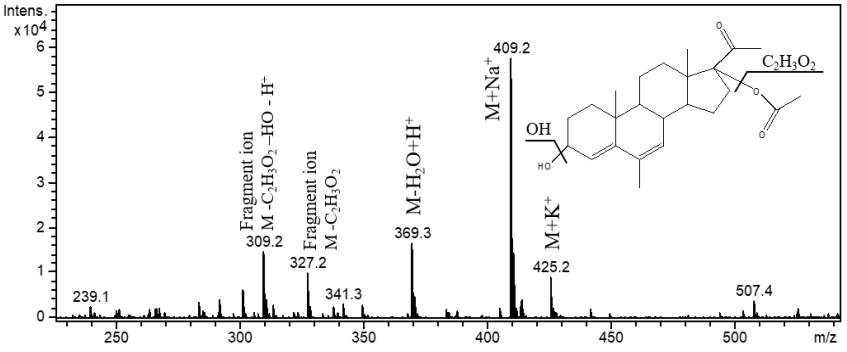
We got good electrospray ionization-MS spectra of metabolite MA with [M+H]+ ions (Pic.1). But the ESI-MS spectra of AMP-17 and GB (Pic.2, 3) showed us ions like [M+Na]$^+$, [M+K]$^+$, and products of GB and AMP-17 fragmentation in the ionization chamber and no ions [M+H]$^+$. Also we found out that the quantification of GB and AMP-17 was impossible because their spectra were unstable and the cationized ions amount depended on eluent.



Pic.1 – ESI-MS-spectrum of MA



Pic.2 – ESI-MS-spectrum of GB

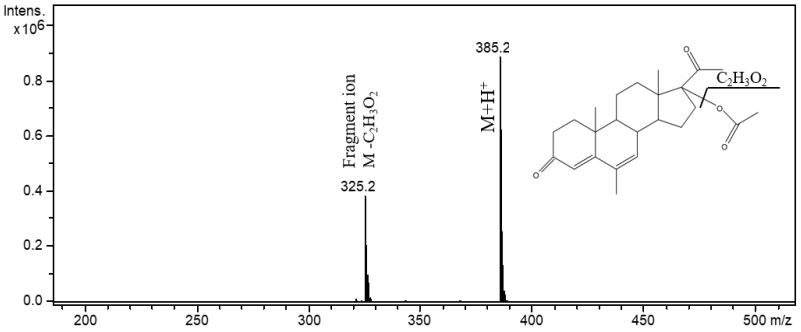


Pic.3 – ESI-MS-spectrum of AMP-17

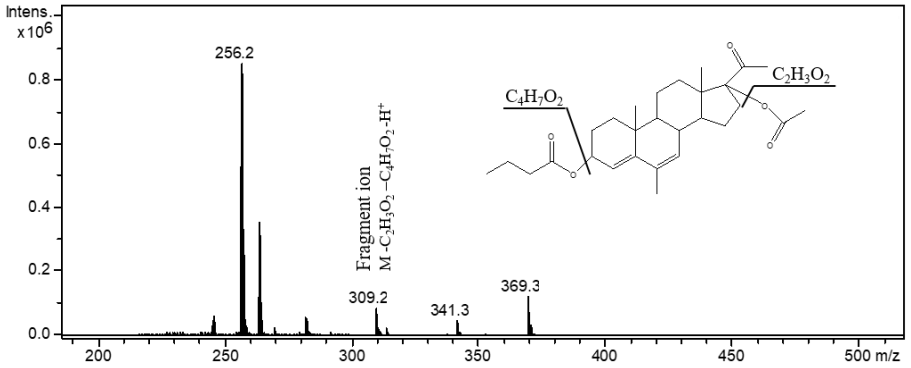
Results and discussion

APCI-MS spectra

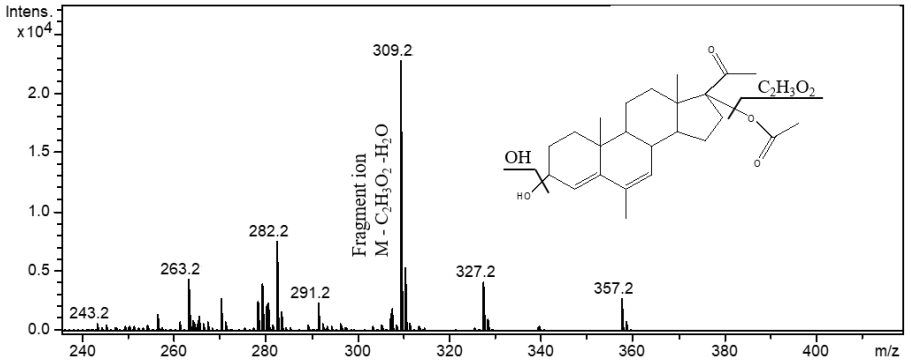
There were no cationized ions in the atmosphere pressure chemical ionization (APCI-MS) spectra. GB and AMP-17 were quantified by protonated fragment ions m/z 309.2 (Pic.4,5). Metabolite MA had $[M+H]^+$ m/z 385.2 ions (Pic.6).



Pic.6 – APCI-MS-spectrum of MA



Pic.4 – APCI-MS-spectrum of GB

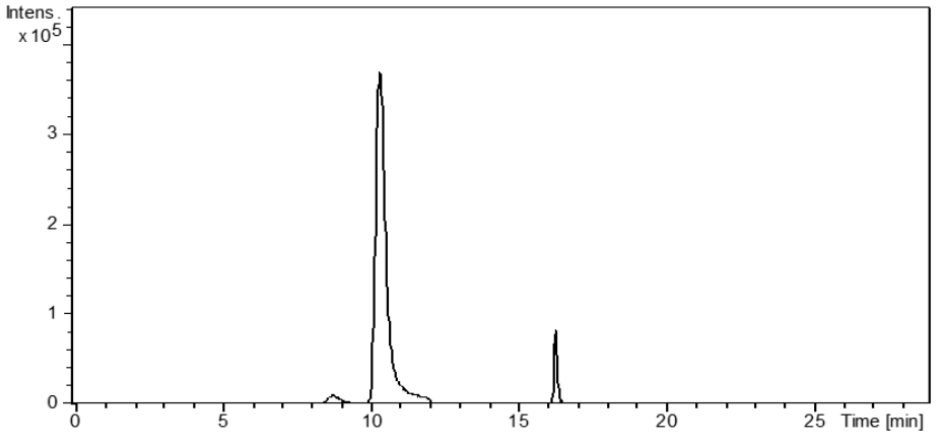


Pic.5 – APCI-MS-spectrum of AMP-17

Results and discussion

HPLC

Column C18 2,1*150 (Thermo) and a stepwise elution mode of water:acetonitrile 1:1 during 0-8 min, then 8-14 min 1:9, from 14min back to 1:1 until 20 min. Injection volume 20 mkl. Under selected chromatographic conditions retention times were 8.6 min, 10.2 min, 16.2 min for AMP-17, MA and GB respectively (Pic.7).



Pic.7 – Chromatogram of working solutions of GB, AMP-17 and MA at one sample.

Sample preparation

The developed HPLC-MS technique didn't require difficult sample preparation. We used easy protein precipitation with two volumes of acetonitrile in relation to the volume of blood serum.

Results and discussion

Method validation

The following parameters were checked out:

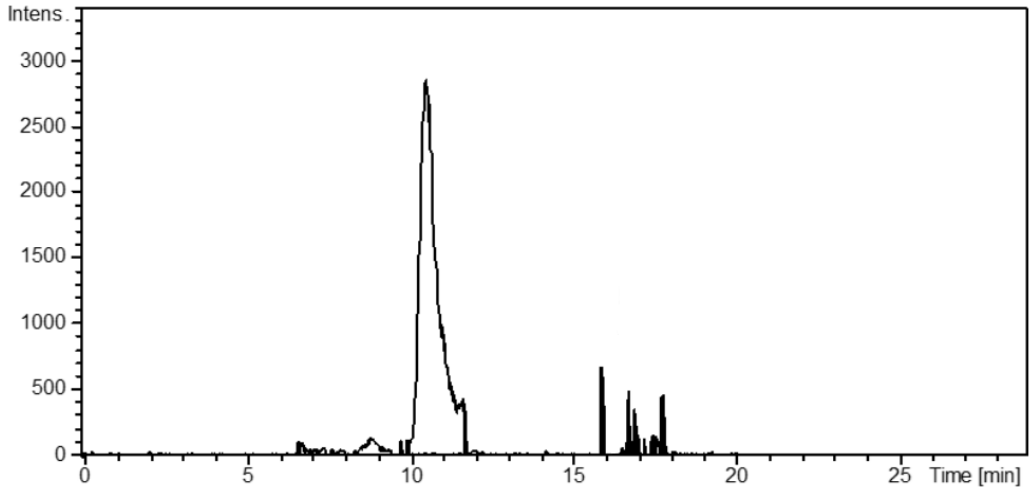
- Selectivity;
- Carry-over;
- Lower limit of quantification – 10 ng/ml for each analyte;
- Calibration curve in the range 10-1000 ng/ml for GB and AMP-17, 10-5000 ng/ml for MA;
- Accuracy;
- Precision;
- Matrix effect;
- Stability of analytes in serum during thawing and freezing;
- Stability of analytes in stock and working solutions;
- Autosampler stability of the processed sample (10°C).

The technique met the requirements of Guideline on bioanalytical method validation (EMA).

Results and discussion

Pharmacokinetics in rats

Female rats weighing 200.0±60.0 g were administered suspension of tablets containing GB (2 mg) in 5% carboxymethylcellulose gel. The dose was 100 mg/kg. For the pharmacokinetics studies rat blood was collected at the time: 0.25. 0.75. 1.5. 3. 6. 12. 24 h after administration. Collected blood was centrifuged to separate serum. Then serum was prepared and analyzed with HPLC-MS. A typical chromatogram is shown in picture 8.



Pic.8 – Chromatogram of working solutions of GB, AMP-17 and MA at one sample.

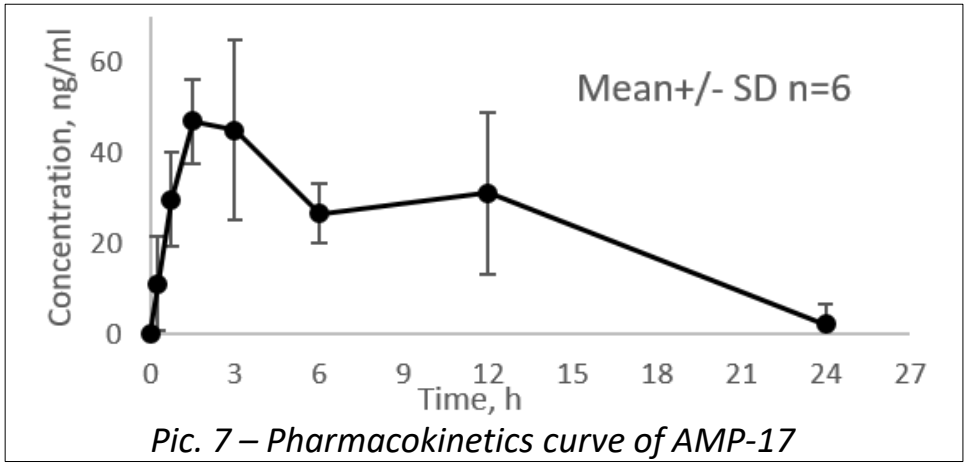
Results and discussion

Pharmacokinetics in rats

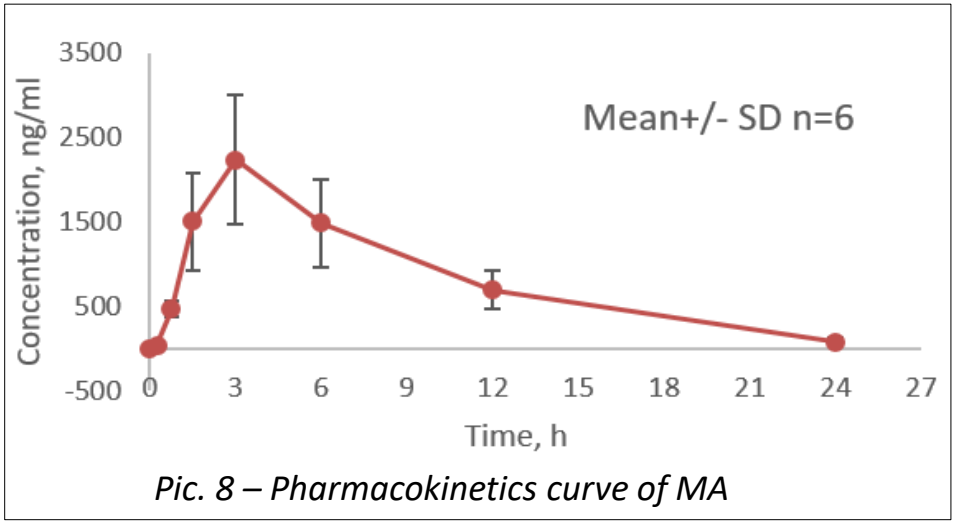
GB wasn't detected in the rat serum during the HPLC-MS analysis. Pharmacokinetics curves for metabolites are presented in the picture 7 and 8.

Time to reach Cmax (Tmax) was 1.5 h for AMP-17 and 3h for MA. Which means the AMP-17 metabolite formed faster.

Cmax of MA significantly exceeded the Cmax of AMP-17 and was 2238.2 for MA and 47.0 for AMP-17.



Pic. 7 – Pharmacokinetics curve of AMP-17



Pic. 8 – Pharmacokinetics curve of MA

Results and discussion

Parameter	AMP-17	MA
AUC_{0-t}, ng/ml/h	590.9	20444.3
AUC_{0-oo}, ng/ml/h	612.1	20978.4
C_{max}, ng/ml ± SE	47.0±4.6	2238.2±379.9
T_{max}, h	1.5	3
CL, l/h	32.67	0.95
K_{el}, 1/h	0.138	0.163
$T_{1/2}$, h	5.0	4.3
MRT, h	8.9	7.7
Vd, l	236.5	5.9

Pharmacokinetics in rats

The distribution volume (Vd) of AMP-17 was about 236.5 liters, which probably indicates an active intake of AMP-17 in tissues and its active biotransformation. For MA, the distribution volume was 5.9 L, which indicates its greater affinity for blood than for tissues, compared with AMP-17. The half-elimination periods ($T_{1/2}$) of metabolites from the blood were almost identical: 5.0 and 4.3 hours for AMP-17 and MA, respectively. The elimination constants for metabolites are also the same and were 0.138 1/hr for AMP-17 and 0.163 1/hr for MA.

Conclusions

The HPLC-APCI-MS method for simultaneous quantification of Gestobutanoil and its two metabolites was developed and validated. Lower limit of quantification for each analyte was 10 ng/ml.

Pharmacokinetics studies of GB tablet dosage (2 mg) in vivo in rats showed that GB is rapidly metabolized into AMP-17 and MA. Pharmacokinetic parameters for metabolites indicate that GB has a stepwise nature of metabolism: the time to reach the maximum concentration of AMP-17 is 1.5 h and MA — 3 h. Metabolite AMP-17 Cmax in serum is far more lower than Cmax of MA. Compared with MA, the metabolite AMP-17 penetrates better into the peripheral tissues.

Also it was shown MA has greater affinity for blood than for tissues, compared with AMP-17.

Acknowledgments

Our researches were supported by the group of scientists of the N.I.Pirogov Russian Medical Research University under the leadership of L.N. Szymanowski.