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Formulation and *in vitro* release of suppositories containing dry extract of *Ruscus aculeatus* L.

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Ruscus aculeatus L. is used in the treatment of venous diseases such as hemorrhoids and capillary fragility. It contains the steroidal sapogenins (ruscogenin and neoruscogenin), and related saponins [1–4]. This paper describes the technological development of suppositories prepared from a spray-dried extract and the assay of ruscogenins by the densitometric high performance thin – layer chromatography (HPTLC) method. The *in vitro* release of ruscogenins from the suppositories and some main biopharmaceutical parameters are also reported.

The suppositories were formulated on the basis of data obtained from the assay of ruscogenins [8]. The single dose – 100 mg IS conformed to similar dosage forms of dry extract of *R. aculeatus* [9]. In order to characterize the suppository formulations, some technological parameters were determined according to the European Pharmacopoeia methods [10].

A comparative analysis of the results obtained in this study shows that Aerosil used as a viscosity promoter in the suppositories enhances the disintegration time, but does not affect the other parameters – melting point and mechanical hardness. The rectal suppositories appeared to be acceptable in terms of the technological requirement [7].

Extracts from the suppositories were obtained using 40% and 80% ethanol with six-fold and five-fold extraction, respectively [7]. Almost complete extraction of ruscogenins was achieved with 80% ethanol – 97% (amount of ruscogenins in 100 mg free extract is 0.4881mg). Therefore 80% ethanol should be used for extracting ruscogenins from suppositories.

The results from *in vitro* release tests of ruscogenins contained in suppositories and some biopharmaceutical parameters are plotted in the Fig. and summarized in the Table.

Hydrophilic Aerosil when incorporated in Witepsol W 35 retards the release of the dry extract, an effect which is directly proportional to the concentration of Aerosil incorporated. This effect may be due to higher viscosity of the suppository mass and/or adsorption of the extract by Aerosil. The results show that the best ruscogenin release rate is obtained with formulations with extract without Aerosil and those with 1% Aerosil.

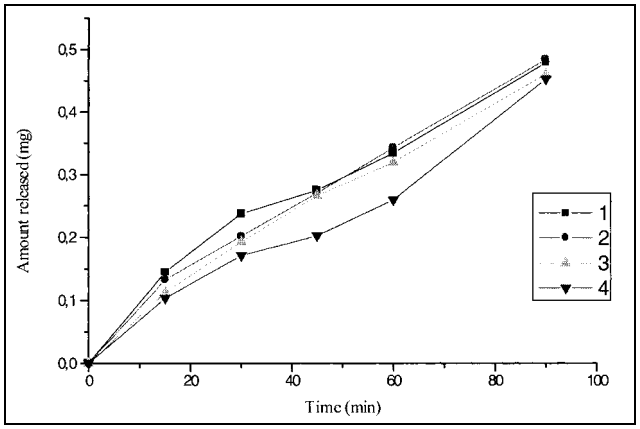

Fig.: *In vitro* release of ruscogenins from suppository models: 1- without aerosil; 2 – with 1% aerosil; 3 – with 2% aerosil; 4 – with 3% aerosil

Table: Mean biopharmaceutical parameters of the suppository formulations

Biopharm. parameters	Model 1	Model 2	Model 3	Model
ABC	3813.67	3941.03	3977.85	4441.28
AUC	5186.32	5058.98	4804.35	4196.03
DE%	57.63	56.21	54.71	48.58
MRT (min)	38.14	39.41	40.77	46.28
VRT (min^2)	796.58	741.02	746.21	835.53
R (min^{-1})	0.55	0.48	0.45	0.39

MRT and VRT determination allows more detailed characterization of the release profiles. A relatively precise definition of the release kinetics can be done using VRT/MRT^2 ratio [5]. In this case the release process follows the law of Hixon – Crowell.

Experimental

1. Materials

Dry extract of *R. aculeatus* L. was obtained from above – ground and underground parts of the plant and standardized following the European Pharmacopoeia [6]. Lipophilic suppository base – Witepsol W_{35} (Dynamit Nobel – Troisdorf – Oberlar – Germany); colloidal silicon dioxide – Aerosil 200 (Degussa – Germany). Ethanol; 1N hydrochloric acid; n-butanol; methanol; ethylacetate – all reagents were of analytical grade (Merck). Chromatographic plate for HPTLC – Fertigplatten Kieselgel 60 10 × 20 cm, Merck-Germany; p-dimethylaminobenzaldehyde test.

2. Preparation and characterization of suppositories

The suppositories were prepared according to the fusion method, using Witepsol W_{35} as base and 100 mg dry extract of R. aculeatus, one formulation without Aerosil and three formulations with 1, 2 and 3% Aerosil respectively [7]. The characterization of suppositories followed the European Pharmacopoeia.

3. Assay of ruscogenin and neoruscogenin in suppositories by the HPTLC densitometric method

Extraction of the dry extract from the suppositories was with 80% ethanol. One suppository was melted in 10 ml ethanol and the mixture was shaken for 5 min. Then the sample was cooled, the suppository mass was separated by filtration and the same procedure was repeated 5–6 times.

The liquid extract was subjected to acid hydrolysis with 1N HCl in the presence of n-butanol. Ruscogenins were determined by densitometric HPTLC on a CAMAG TLC scanner II at 520 nm [8].

4. In vitro drug release

In vitro release of extract was performed in a Vibroterm shaker bath at 37 °C, 100 rpm, in 100 ml phosphate buffer (pH = 7.4). Samples of 2 ml were periodically pipetted out from the vials and replaced by the same volume of fresh medium. After hydrolysis, the amount of ruscogenins in the released extract was determined by the densitometric HPTLC method (3).

References

1 Bombardelli, E.; Bonati, A.; Gabetta, B.; Mustich, G.: Fitoterapia **42**, 127 (1971)
2 Bombardelli, E.; Bonati, A.; Gabetta, B.; Mustich, G.: Fitoterapia **43**, 3 (1972)
3 Moura, T.; Gaudy, D.; Casadebaig, J.; Jacob, M.: Pharmazie **50**, 752 (1995)
4 Mauss, H. J.: Phlebologie **21**, 200 (1992)
5 Minkov E.; Lambov, N.; Ovtcharov, R.; Bantutova, I.: Osnovi na biopharmaciata, Venel Medic Ed., Sofia 1994
6 Nikolov, S.; Krasteva, I.; Peneva, B.; Minkov, E.: Pharmacia (S) **44**, 11 (1997)
7 Peneva, B. I.; Krasteva, S.; Nikolov, M.; Padulova; Minkov, E. N.: Pharmacia (S) **46**, 16 (1999)
8 Peneva, B.; Krasteva, I.; Nikolov, S.; Minkov, E.: Pharmazie **55**, 7 (2000)
9 Rote Liste – ECV " Editio Cantor" – Aulendorf/Württ. 1996
10 Zuber, M.; Pellion, B.: S.T.P. Pharma **3**, 593 (1987)

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