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Escin in pharmaceutical oral dosage forms: quantitative densitometric HPTLC determination

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Dedicated to my father

Abstract

A practical and reliable method for the quantitative determination of escin in pharmaceuticals was developed. The TLC–densitometric determination was performed without using spray or dipping reagents in order to provide a more rapid and simple analytical procedure. Chromatographic separations were obtained on analytical HPTLC silica gel plates and the elution was made with 3.5:4.5:2 PropOH–ethyl acetate–water (v/v/v). Densitometric analysis was performed directly at 212 nm, corresponding to λ_{max} of escin obtained by in situ-scanning. A second degree polynomial regression relationship was found between the peak areas and the amounts of the escin standard deposited in the range 1.15–6.90 μg. The method is specific, accurate and reliable and was applied successfully to the quantitative determination of escin in commercial samples.

Keywords: Escin; Thin-layer chromatography; Densitometry; Analysis; Oral dosage forms

1. Introduction

Escin is a natural mixture of triterpene saponins occurring in the seeds of the horse chestnut (*Aesculus hippocastanum* L.). The structures of two major glycosides present in the mixture are reported in Fig. 1. Their aglicons are derivatives of protoascigenin acylated by the acetic acid at C-22 and by either angelic or tiglic acids at C-21 [1]. Because of its anti-inflammatory, capillaro-protective and antiedematous properties, and because of its high degree of tolerability, escin is largely employed in the therapy of peripheral vascular disorders [2].

Escin has proved to be effective in the localised treatments of edema, phlebitis, post-thrombotic syndrome and in the remission of the ulcerous symptoms in patients who are subject to current thrombophlebitic attacks. Furthermore, it has also been used in cerebral edemas and intracranial aneurisms [3,4], in the resolution of the edematous state in gynecology [5], and in odontotherapy [6] to improve gingival haemorrhages. The vasoprotective action of escin also constituted the

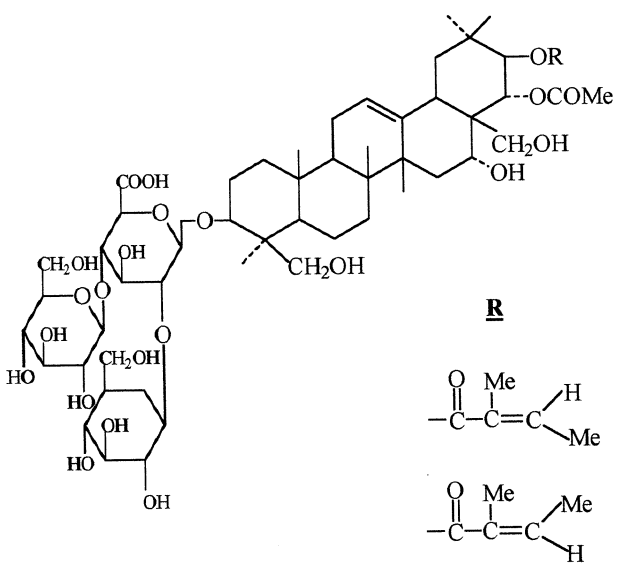

Fig. 1. Principal saponins of escin.

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basis for its application in dermatology, particularly in the treatment of chronic venous stasis and for the prevention/treatment of panniculopatia edemato-fibroschlerotica, so-called cellulitis [7].For the determination of escin colorimetric analysis [8], enzyme immuno assay [9], radioimmunoassay [10], TLC–densitometric [11–13] and HPLC methods [12,14–16] have been proposed. Nevertheless, this work describes a new application of TLC–densitometry for direct determination of escin in pharmaceutical oral dosage forms without derivatising agents.

2. Experimental

2.1. Chemicals and samples

A reference standard of escin was obtained from Fluka (Buchs, Switzerland). The investigated pharmaceutical products were commercial samples, containing escin (with dosages of 40 and 20 mg), hesperidin and various excipients, like lactose, carbowax 1500, carbowax 6000, magnesium stearate, saccarose etc. The solvents and other chemicals were obtained from Farmitalia Carlo Erba (Italy). The filters were obtained from Whatman (NJ, USA).

2.2. Equipment

Camag Linomat IV was used as the application device. A horizontal developing chamber and a TLC scanner II were also provided by Camag (Muttenz, Switzerland). The TLC scanner was connected, by means of an interface, with Merck–Hitachi Model D-7000 chromatography data station software, version 3.0 (E. Merck, Darmstadt, Germany).

2.3. Procedure

2.3.1. Preparation of the standard solution

The standard solution was prepared through dissolution of 11.50 mg of escin in 10 ml of methanol, to obtain a concentration of 1.150 mg/ml. The solutions were stored at 4°C under nitrogen in the dark, and freshly prepared each week.

2.3.2. Preparation of the sample solutions

A quantity of powdered tablets, corresponding to 50.0 mg of escin, was suspended in 50.0 ml of methanol and agitated for 20 min in an ultrasonic bath. The suspension was filtered through a membrane filter of 0.45 μm. The filtrate was directly deposited on the plate together with the standard solution for quantitative determination.

2.3.3. HPTLC–densitometry

Chromatography was performed on analytical HPTLC silica gel 60 F_{254} plates (10 × 10 cm) of 0.2 mm thickness (E. Merck, Darmstadt, Germany). Samples (4 μl) and standard solutions (1–6 μl) were applied to the plate as 8 mm bands (10 mm from the lower edge of the plate) by means of a Camag Linomat IV applicator (rate of delivery 10 s/μl; 7 bands per plate). For each analysis, one single plate contained six levels of standard and one of a sample. The plates were developed for about 6.5 cm from the baseline in a saturated horizontal developing chamber with 3.5:4.5:2 PropOH–ethyl acetate–water (v/v/v). Developed plates were dried with a stream of lukewarm air and quantified by linear scanning in reflectance mode at 212 nm, by means of a computer assisted (D-7000 Chromatography Data Station Software) Camag TLC-scanner. The spots were analysed using the following parameters: single-beam reflectance mode; monochromator bandwidth 10 nm (micro position); slit dimension 0.3 × 4 mm; scanning speed 0.5 mm/s; automatic zeroing before each track; automatic sensitivity adjustment.

The 'net chromatograms' of each analyte (standards and samples) were obtained subtracting via computer, in the same plate, the registered signal of a blank track from the signal of the analyte.

3. Results and discussion

This work was aimed at finding a method for the quantitative determination of escin in pharmaceuticals.

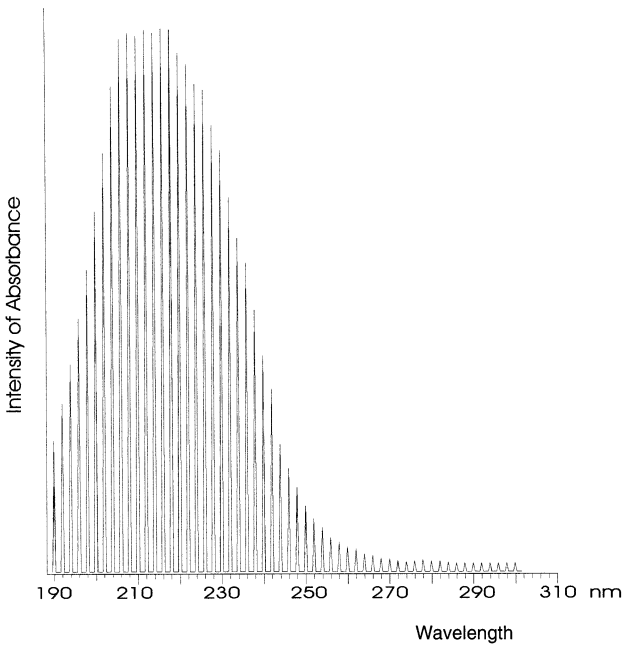

Fig. 2. Absorption spectrum of escin ($\lambda_{max} = 212$ nm) on silica gel plate.

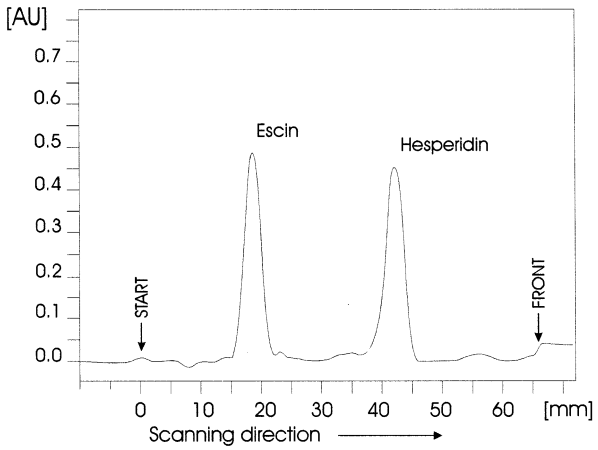

Fig. 3. Densitometric reflectance mode scanning profile of HPTLC chromatogram of a sample solution at 212 nm on silica gel.

The co-presence of substances absorbing in the UV region (such as hesperidin in the samples analysed) realises the use of a chromatographic technique. The TLC–densitometric method is applicable and advantageous for time saving and cost effectiveness.

Unlike previous works [12,14,15,17] the TLC–densitometric determination was performed without using spray or dipping reagents in order to provide a more rapid and simple analytical procedure.

The densitometric analysis was performed directly at 212 nm, corresponding to λ_{max} of escin obtained by the in situ-scanning of a standard solution (Fig. 2).

This absorbance spectrum was obtained by scanning in the range 190–300 nm. When measuring below 200 nm, oxygen in the air absorbs some UV light, diminishing light yield and consequently the sensitivity of the measurement. To avoid this, the optical system and the measuring compartment were purged with nitrogen.

A densitogram of a sample containing escin and the excipient hesperidin is presented in Fig. 3. The R_f value for escin was 0.28 (RSD = 1.35).

In TLC–densitometry the main difficulty in increasing the sensitivity is the noise component of the signal associated with heterogeneity of the layer structure and the migration of the solvent with elution. There are works in the literature indicating that the background subtraction routines provide a significant increase in sample determination [17,18].

It is preferred to examine a chromatogram that is representative of only the analyte, free from the background. In fact, it is desirable to subtract the background signal from the scan that contains the chromatograms of both the analyte and the background. This sequence is represented in Fig. 4. The top panel (a) of Fig. 4 shows the combination of the chromatogram of the standard analyte plus the chromatogram of the background. The middle panel (b) represents the recorded signal of a blank track registered after elution, due only to the background. The computer operated connection, by means of an interface, of the exit signal from TLC-scanner with the chromatography data station software made it possible to subtract scan b from scan a. The result of this subtraction is shown in the bottom panel of Fig. 4, which is the 'net chromatogram'.

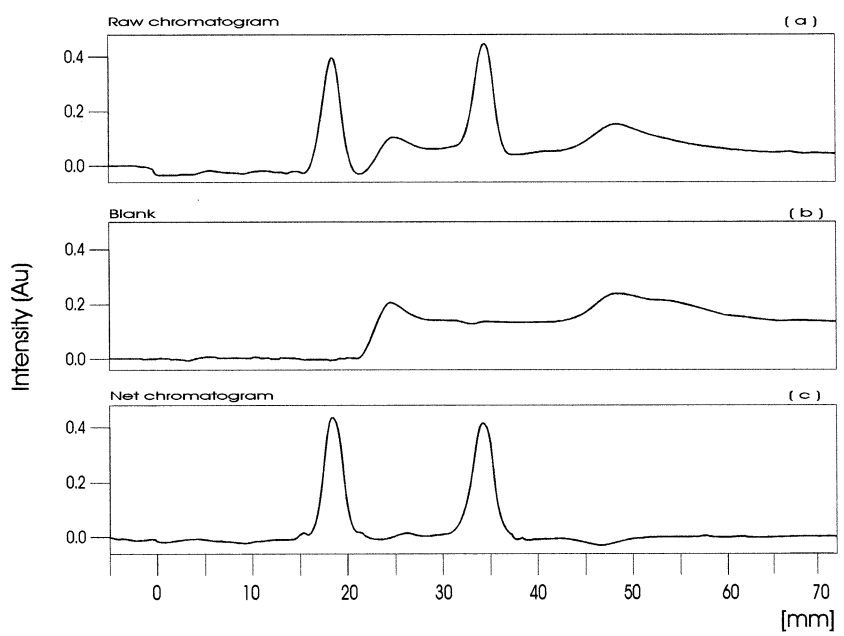

Fig. 4. The top panel (a) shows the combination of the chromatogram of the standard analyte plus the chromatogram of the background. The middle panel (b) represents the recorded signal of a blank track registered after elution, due only to the background. The bottom panel (c) shows the 'net chromatogram', obtained subtracting, via computer, (b) from (a).

Table 1
Assay results for the HPTLC densitometric determination of escin in commercial preparations [a]

Preparation (tablets) [b]	Theoretical content of escin (mg)	Amount of escin found		
		$\bar{x}$(mg)	Found (%)	RSD (%)
A	40	39.84	99.6	1.42
B	40	41.08	102.7	2.10
C	40	40.20	100.5	1.83
D	20	20.28	101.4	1.66
E	20	19.74	98.77	1.95
F	20	20.14	100.7	1.78

[a] Volume of sample solutions applied: 4 μl. The results given are the means of six determinations.
[b] Components of the preparations A, B, C: escin, hesperidin, lactose, saccarose, talc, carbowax 1500, carbowax 6000, magnesium stearate, arabic gum, eudragit L, castor oil, silicic acid, titanium dioxide. Components of the preparations D, E, F: escin, hesperidin, lactose, calcium carbonate, talc, carbowax 1500, carbowax 6000, magnesium stearate, eudragit L, castor oil, silicic acid, arabic gum, saccarose.

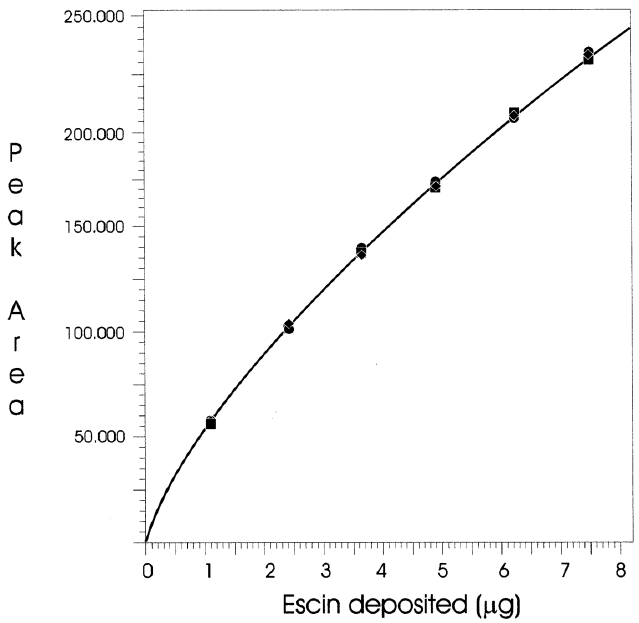

Fig. 5. Peak area vs. amount of escin deposited.

Because TLC plates are strongly light-scattering, the absorption signal does not obey Beer's law: it is usually only pseudolinear at low sample concentration and the quantification is generally based on calibration in which a second-order polynomial fit of the calibration curve is used for interpolation of the concentration of the unknown samples [18]. In agreement with this, a second degree polynomial regression relationship was found between the peak areas and the amounts of the escin standard deposited in the range 1.15–6.90 μg (Fig. 5). The calibration points were obtained in triplicate at six levels applying 1–6 μl of the standard solution. The corresponding correlation coefficient (r) was found to be greater than 0.9988. The limit of detection (LOD) and the limit of quantification (LOQ), calculated as the analyte concentrations that produce a chromatographic peak three and ten times higher than the noise level, were 0.27 and 0.90 μg, respectively.

The precision of multiple scans of the same spot was found to be less than 0.92% relative standard deviation (RSD) at all levels of the calibration curve. The inter-assay precision (six replicates and three scanning runs) was found to be less than 2.10% RSD.

Accuracy was assessed adding quantified escin standard to micronised powdered tablets and suspending with methanol, to obtain 80, 100 and 120%, respectively, of the target concentration of escin analysed. For these three levels the recovery obtained was $99.55 \pm 1.14\%$ (RSD) ($n = 6$), $100.13 \pm 0.33\%$ (RSD) ($n = 6$) and $100.54 \pm 0.51\%$ (RSD) ($n = 6$), respectively. The analytical results for the investigated preparations are reported in Table 1.

4. Conclusions

The results indicate that the method developed is simple, rapid and can be used for the quality control of pharmaceutical preparations containing escin. The above mentioned chromatographic method showed appropriate selectivity to obtain the specific determination of escin, without interference due to the contingent co-presence of substances absorbing in the UV region.

The background subtraction routines by means of software allow one to improve the chromatograms and to obtain quantitative results with excellent precision and accuracy.

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References

[1] G. Wulff, R. Tschesche, Triterpenes. XXVI. On the structure of the horse-chestnut saponin (aescin) and the aglicones of various glycosides, Tetrahedron 25 (1969) 415–436.

[2] M.H. Pittler, E. Ernst, Horse-chestnut seed extract for chronic venous insufficiency. A criteria-based systematic review, Arch. Dermatol. 134 (1998) 1356–1360.
[3] S. Mingrino, M. Scanarini, Anti-edema effect of escin at the cerebral level, Minerva Anestesiol. 44 (1978) 355–360.
[4] R. Hemmer, Therapy of brain edema in craniocerebral trauma, Der Unfallchirurg 88 (1985) 93–96.
[5] A. Tolino, Medical treatment of the anorectal varicose syndrome in gynecology and obstetrics, Minerva Ginecol. 31 (1979) 169–174.
[6] G.L. Bertrand, Experimental study of beta-aescine (1) gel in oral and periodontal surgery, Rev. Odontostomatol. Midi Fr. 39 (1981) 211–216.
[7] G. Proserpio, S. Gatti, P. Genesi, Cosmetic use of horse-chestnut (*Aesculus hippocastanum*) extracts, of escin and of the cholesterol/escin complex, Fitoterapia 51 (1980) 113–128.
[8] E. Von Kammerl, W. Schlemmer, Automated chemical determination of escin in horse-chestnut extract and in pharmaceutical preparations, Pharm. Ind. 34 (1972) 356–358.
[9] C. Hentschel, W. Schossler, G. Liebrich, A Brattstrom, Enzyme immuno assay for quantitative determination of beta-aescin in human serum, Pharmazie 49 (1994) 929–930.
[10] T. Lehtola, A. Huhtikangas, Radioimmunoassay of aescin, a mixture of triterpene glycosides, J. Immunoass. 11 (1990) 17–30.
[11] E. Uberti, E.M. Martinelli, G. Pifferi, TLC–densitometric analysis of aescin in ointments, Fitoterapia 61 (1990) 57–60.
[12] O. Mete Kockar, K. Mustafa, K. Serap, B. Berrin, Baser K. Husnu, Quantitative determination of escin. A comparative study of HPLC and TLC–densitometry, Fitoterapia 65 (1994) 439–443.
[13] M. Vanhaelen, R. Vanhaelen-Fastre, Quantitative determination of biologically active constituents in medicinal plant crude extracts by thin-layer chromatography–densitometry. I. *Aesculus hippocastanum* L., *Arctostaphyllos uva-ursi* Spreng, *Fraxinus excelsior* L., *Gentiana lutea* L., *Glycyrrhiza glabra* L., *Hamamelis virginiana* L., *Hypericum perforatum* L., *Olea europea* L., *Salix alba* L. and *Silybum marianum* Gaertn, J. Chromatogr. 281 (1983) 263–271.
[14] P. Pietta, P. Mauri, R. Maffei, M. Carini, High-performance liquid chromatography analysis of β-escin, J. Chromatogr. 478 (1989) 259–263.
[15] B. Renger, Quantitative densitometric HPTLC determination of triterpenic saponins (escin) in horse-chestnut extracts, J. Planar Chromatogr. 3 (1990) 160–162.
[16] A. Griffini, E. Lolla, F. Peterlongo, Liquid chromatography–thermospray mass spectrometry analysis of beta-escin, Fitoterapia 68 (1997) 520–526.
[17] R.E. Kaiser, Modern quantitation in planar chromatography, J. Planar Chromatogr. 4 (1989) 323–326.
[18] C.F. Poole, S.K. Poole, Instrumental thin-layer chromatography, Anal. Chem. 66 (1994) 27–37.