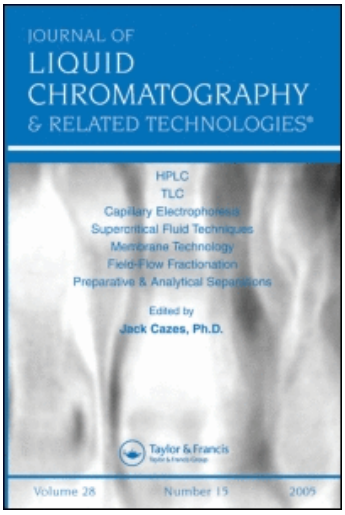

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ESTABLISHMENT OF XYLOSE IN *PLANTAGO OVATA* FORSSK. AS A LEADING COMPOUND FOR QUANTIFICATION IN RAW MATERIAL AND FINISHED PRODUCT

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□ *A fast and accurate densitometric method was developed and validated to quantify Plantaginis ovatae semen (also: 'ispaghula seed') in Agiocur®. Ispaghula seeds have a long history of use as a laxative, but still there was no method for the quantification in the medicinal product. Because of the increasing number of guidelines for medicinal products, it was necessary to develop an appropriate method. The selected leading-substance, xylose, in ispaghula seed is the main sugar in the mucilage layer of the seed husks. This mucilage produces the laxative effect by acting mainly mechanically. The effect starts when the seeds come into contact with water, and the mucilage polysaccharides begin to swell. The analytical method is based on the densitometric evaluation of the TLC-plate after the second development with the mobile phase acetonitrile-water (90:10, v/v). The xylose zone occurs at an Rf-value of 0.56. Xylose is completely separated from the other sugars in the hydrolysate. The derivatisation reagent 4-aminobenzoic acid is used to color xylose to a red-brown color. The evaluation in the TLC-scanner is executed by using the peak height at 366 nm. The recovery from xylose in the finished-product-excipient (placebo) mixture is between 102 and 106 percent. The precision of a six-time preparation of the hydrolysate from the seeds of a regular batch amounts to a relative standard variation (RSD) of 1.59 percent. As a result, Agiocur® contains 140 mg/g xylose delivered by the P. ovatae semen.*

Keywords Agiocur®, densitometric analysis, *Plantago ovata* forrsk., plantaginis ovatae semen (ispaghula seed), plantaginis ovatae seminis tegumentum (ispaghula husk), TLC, validation

INTRODUCTION

For decades, Agicour® from Madaus has proven itself to be a beneficial herbal drug which supports the human digestive system.[1]

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As key ingridient, Agicour® contains ispaghula seeds and ispaghula husks from *Plantago ovata* Forssk. This plant is mainly being cultivated in India and Pakistan.[2,3]

It belongs to the "Plantaginaceae" family and has been traditionally used to treat sickness of the human digestive system.[1]

The pharmacological effect is based on a singular polysaccharide structure located mainly in the bowl of the seeds, which swells upon contact with water. The seed bowl consists of 95% of the effective mucilage polysaccachrides.[4–7] These mucilage polysaccharides increase the viscosity of the feces upon uptake and possess hence a way laxative effect due to mechanical effects.[8,20,21] Another positive effect is the cholesterol lowering effect which is caused by the binding of bile acids and cholesterol of the swollen mucilage in the small intestine.[26] The polysaccharides also may express specific stimulation of growth factors which are involved in the proliferation.[27] Other active ingredients are fatty oils,[18] ß-sitosterol,[28] and, in small quantities, the iridoid glycosides aucubin and catalpol, as well as other flavonoids and CPGs.[8,25] So far, the swelling capacity and the composition of sugars after the hydrolysis were used to demonstrate ispaghula seed in the final product.[9] In the German Pharmacopoeia, it is required that the ripe seeds must have a swelling index of at least 9.[16] However, the swelling index varies upon material preparation and, thus, makes no precise statement on the content of the seeds in the product. For this reason, a densitometric determination of xylose as a leading compound has been established. Xylose, arabinose, and galactose are the main sugars in the mucilage layer.[7,17–19]

EXPERIMENTAL

Chemicals and Plant Material

The plant material was delivered by the Madaus Company from India. The xylose standard (2462972/Roth) was ordered from Roth (Roth, Karlsruhe, Germany). The solvent methanol (K38323409/Merck), the barium carbonate for neutralisation and the 4-aminobenzoic acid for the proof reagent were purchased from Merck (Darmstadt, Germany). Phosphoric acid 85%, also needed for the proof reagent, was ordered from Riedel de Haen (Hanover, Germany), Acetone from Sigma Aldrich (Munich, Germany) and water-free acetic acid from Merck (Darmstadt, Germany). The acetonitrile (73455/Riedel de Haen) used as mobile phase was ordered from Riedel de Haen.

Preparation of the Plant Material

1.5 g ground test substance is dissolved in 60 mL 4% sulfuric acid *R*, v/v, shifted into a 100 mL Erlenmeyer flask and heated for 90 minutes under

reflux. After cooling down, the extract is clearly filtered over foldedfilter (595 ½). 20 mL brownish filtrate is neutralised with barium carbonate *R* (attention strong foaming strength), whereby a milky suspension develops. To shorten the waiting period which is necessary to let the foam settle, the suspension is briefly centrifuged and afterwards filtered again. Two milliliters of filtrate are taken and filled up with methanol to 100 mL.

Preparation of the Standard Solution

For the stock solution, 20 mg xylose is weighed into a 100 mL round-bottom flask and filled up with methanol. For the standard solution, the stock solution is diluted with methanol, so that six standard concentrations from 40 to 120 µg/mL xylose are prepared.

Chromatography

For the thin layer chromatography, TLC silicagel plates (20 × 20 cm) with 0.25 mm layer thickness from Merck (Merck HX890027) were used. The samples and standard solutions were *up-sprayed* with semiautomatic order equipment Linomat IV with nitrogen gas, with 10 mm bandwith at a distance of 8 mm. The standard and sample solution volume of 5 µL was applied with a 100 µL micro litre syringe. The positioning of the spots begins with an initial position of 25 mm and 15 mm distance from the lower disk edge with an application speed of 6 µL/sec. Acetonitrile-water (90:10, v/v) served as mobile phase. The development took place with chamber saturation at ambient temperature with double development with a separating distance of 15 cm and intermediate drying in a cool air stream for 20 minutes.

The development took place in a Desaga developing chamber with glass cover. Subsequently, the TLC plates were dipped for 1 to 2 seconds into 4-aminobenzoic acid reagent. For the derivatisation, the plate is put for 10 minutes under observation on a 110°C warm heating plate. After the colour formation of the sugar zones, the plate was scanned in the DC scanner by Camag with the "wincats 3" software at a wavelength of 366 nm. As light source deuterium light was used. A slit dimension of 6 × 0.2 mm, scanning speed of 20 mm/s and a data dissolution of 100 µL/step were applied. The evaluation was made via the peak height with an external calibration. For the calibration, three reference standards were prepared, which covered the working range.[12]

RESULTS AND DISCUSSION OF THE VALIDATION

The five-point-calibration over the working range from 40 to 120 µg/mL xylose showed a polynomial regression. The linearity regression coefficient

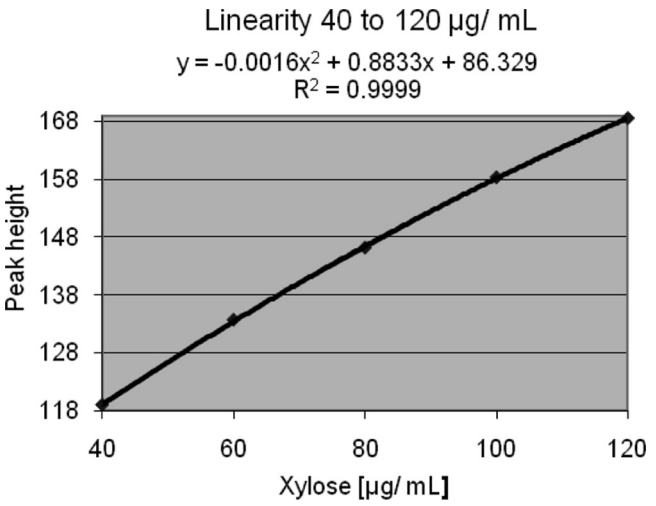

FIGURE 1 Linearity xylose from 40 bis 120 µg/mL.

was $R^2 = 0.9999$ (Figure 1). To avoid the use of complex polynomial functions, quasi-linear calibration functions can be obtained in limited concentration ranges. In routine work, this is approached in the form of a 3-point calibration.

Accuracy

The testing of possible interference of the excipients mixture in the finished drug with the leading substance showed that no xylose is contained in the excipients mixture (Figure 2).

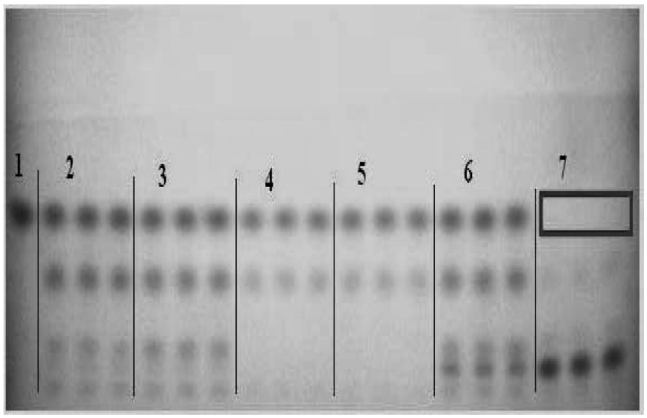

FIGURE 2 DC specifity; key from the left side 1: xyloserefence, 2: ispaghula seed, 3: verified ispaghula seed, 4: ispaghula husk, 5: verified ispaghula husk, 6: Agiocur, 7: auxiliary material mixture from agiocur.

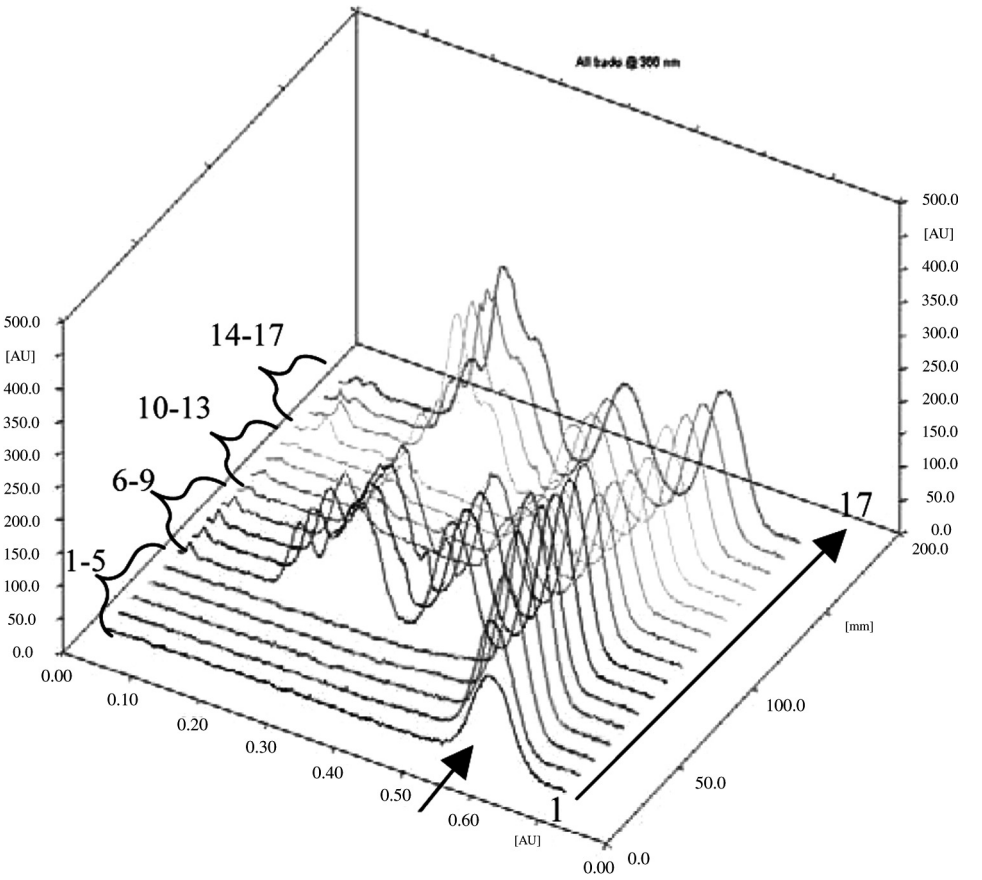

FIGURE 3 DC-chromatogram; 1–5: xylose-reference standards; hydrolyzate 6–9: ispaghula seed; 10–13: ispaghula husk and 14–17: Agiocur.

The specific sugar fingerprint of the ispaghula seed can be compared with the fingerprint of the finished drug (Figure 3). Over the external xylose calibration, the content of xylose in ispaghula seed, ispaghula husk, and in Agiocur can be determined.[11]

The testing of the homogeneity over different batches took place by analysing several batches of Agiocur®, ispaghula seeds, and ispaghula husks. As evaluation criterion, the relative standard deviation of the xylose content was accounted for (Figures 4 and 5).

There were greater variations in the levels of ispaghula seed when they had been milled with a specific mill (batches: I2009032 up I200934) (Figure 4). In the examination of nine different Agiocur® batches, slight fluctuations were observed. It should be noted that trial batches of retained samples, which have been stored at different times after the production,

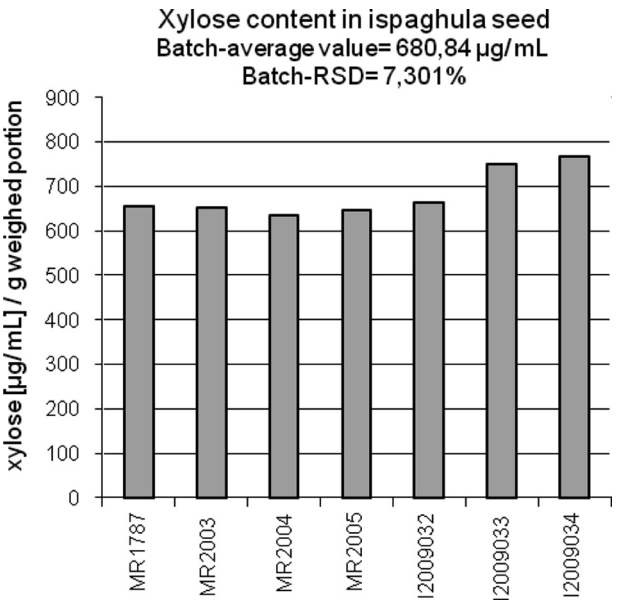

FIGURE 4 Batch-homogeneity of ispaghula seed.

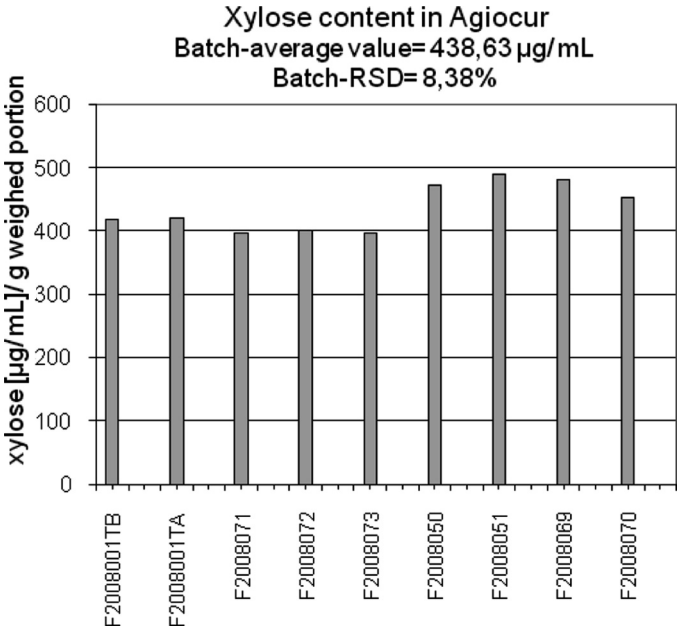

FIGURE 5 Batch-homogeneity of agicour.

TABLE 1 Accounting of Xylose in Agiocur. Data are Averaged Values from the Study of the Batch Homogeneity

1 g Ispaghula Seed	1 g Ispaghula Husk	
Xylose [µg/mL]	Xylose [µg/mL]	
681	1821	
65 g Ispaghula Seed	2 g Ispaghula Husk	100 g *Agiocur*
Xylose [mg/mL]	Xylose [mg/mL]	xylose [mg/mL]
44.2	3.64	43.8
	Theoretical-value xylose in *Agiocur* [mg/mL]	47.84
	Real-value xylose in *Agiocur* [µg/mL]	43.8
Balance Sheet Total [w/w %]		
91.55		

were used (Figure 5). However, a decrease in xylose concentration in relation to the storage time of the batch could not be observed.

The xylose content in the finished product shows a linear correlation with the content of xylose in the ispaghula seeds and the ispaghula husks, which are additionally added. This correlation is displayed in Table 1. According to the manufacturers information, 100 g Agiocur® should contain 65 g ispaghula seeds and, additionally, 2 g ispaghula husks. To determine if the theoretically expected content of xylose was also present in the finished product, the amount of xylose contained in ispaghula seeds and the ispaghula husks was compared with the actual content of xylose in Agiocur® (see Table 1). The average values of the examined batches were used for the theoretical calculations (Figure 5).

The precision with the sixfold processing of the same finished drug batch was evaluated via the relative standard deviation which was not allowed to be more than 5 percent (see Table 2).

The recovery was examined with the increasing method with xylose standard. Hence, a certain quantity of xylose was added to the hydrolysates of the ispaghula seeds, ispaghula husks, and the finished drug excipient material mixture (Table 3).

In the context of the robustness examination, the TLC development was only accomplished once, which led to deviations due to the incompletely separated sugar zones. Besides, the mobile phase composition was varied.

TABLE 2 Results of Precison (n = 6) of Ispaghula Seed, Ispaghula Husk and Agiocur

	Default Precision (%) RSD	Precision (%) RSD
Ispaghula Seed	<5%	2.18
Ispaghula Husk	<5%	4.91
Agiocur	<5%	1.59

TABLE 3 Results of Validation: Recovery Were Tested with the Increasing Method. Thereby the Hydrolyzate of Ispaghula Seed and Ispaghula Husk Increased with Xylose Reference

Increasing Method	Recovery (n = 3) (%)
Ispaghula Seed	
20%	100.43
40%	105.69
60%	101.59
Ispaghula Husk	
20%	99.16
40%	99.12
60%	105.78
Agiocur Placebo	
20%	105.27
40%	103.56
60%	102.39

CONCLUSIONS

The analysis of xylose with DC scanner can be used for quantifying ispaghula seed and ispaghula husk in the finished drug. The measurement is repeatable and reveals consistent quantities of xylose in several batches. The content of xylose in ispaghula seed can be specified to at least 180 mg/g. The contained xylose of the ispaghula seeds and ispaghula husk is recovered in the finished product and can be used as a leading substance. The acquired data document that the presented quantitative, densitometric method meets all requirements for an accurate validation.[22,23]

The results of the validation indicate that the method can be used in routine analytics of pharmaceutical companies for quantifying ispaghula seeds and ispaghula husks in pharmaceutical preparations.

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