INFLUENCE OF VISCOSITY ON DROPLET SIZE DISTRIBUTION IN NASAL SPRAYS

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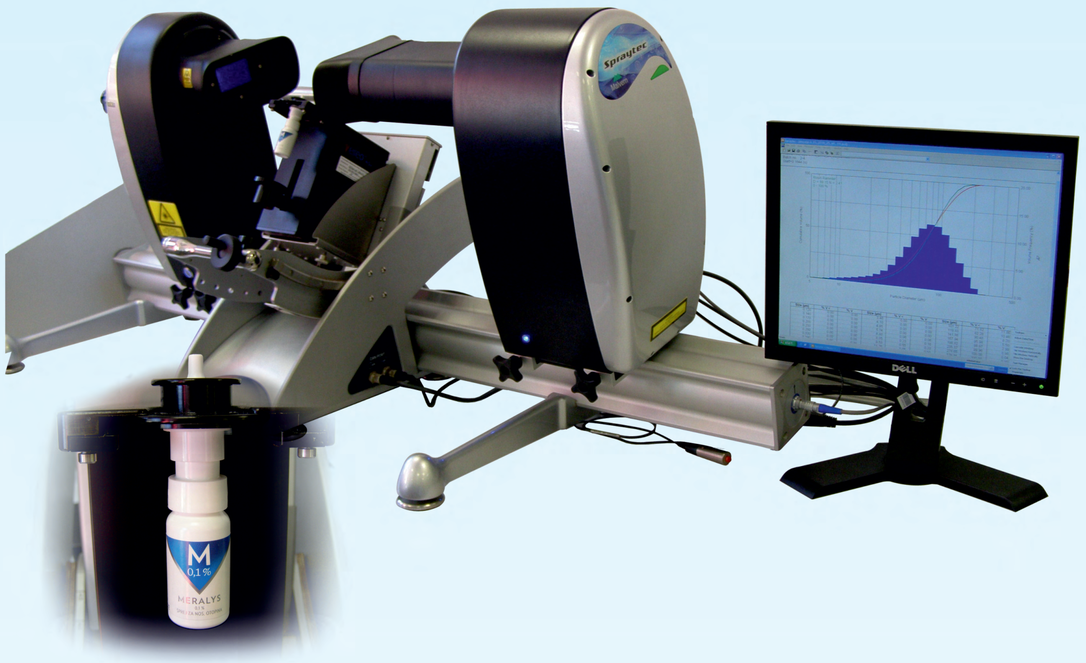
INTRODUCTION

Optimal deposition of drug in nasal passages is an important feature of nasal sprays. Droplet size distribution (DSD) is one of the characterization parameters that could provide information on consistency of the spray and regular deposition in nasal passages. It should be demonstrated that deposition of the product is localized in the nasal cavity, i.e. by demonstrating that the vast majority of the droplets are larger than 10 microns [1]. Addition of excipients may have a significant effect on the properties of the spray plume, affecting the deposition site of the aerosol [2]. One of the primary parameter affecting droplet size distribution is viscosity. During the pharmaceutical development of a generic product with application trough a spray pump, DSD value similar to the reference product could be obtained by testing a range of solutions with different viscosities and comparing droplet size distribution parameters as an in vitro bioequivalence parameter.



METHOD

DSD was measured using a Malvern Spraytec instrument, and in order to obtain reproducible and comparable results, actuation was performed automatically using a Vereo NSx actuator from Proveris Scientific, since it was confirmed that automated actuation reduces variability in the results as oppose to manual actuation [3].

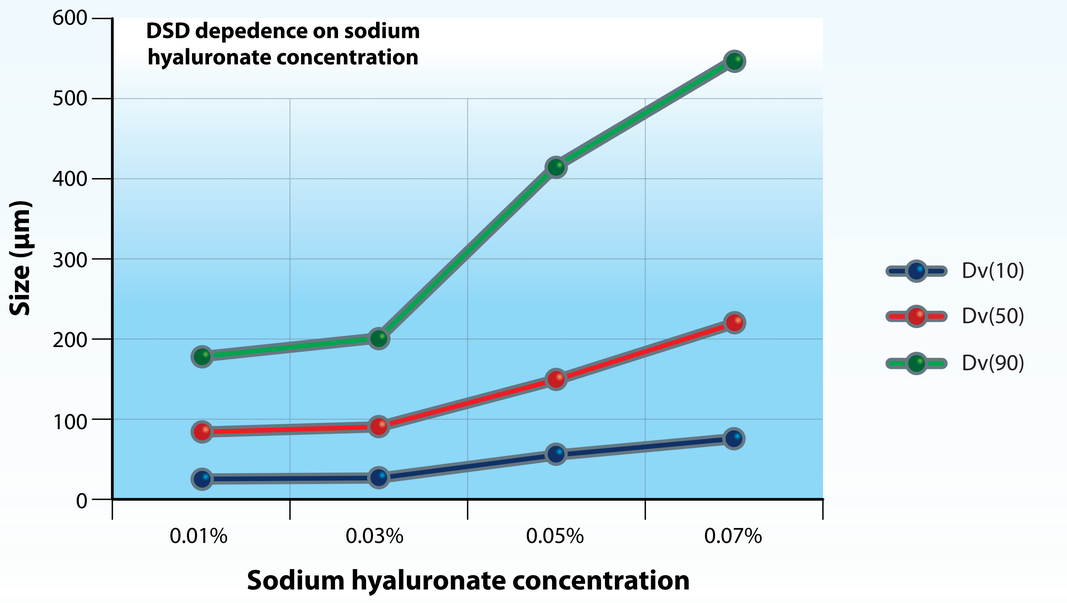
SAMPLES

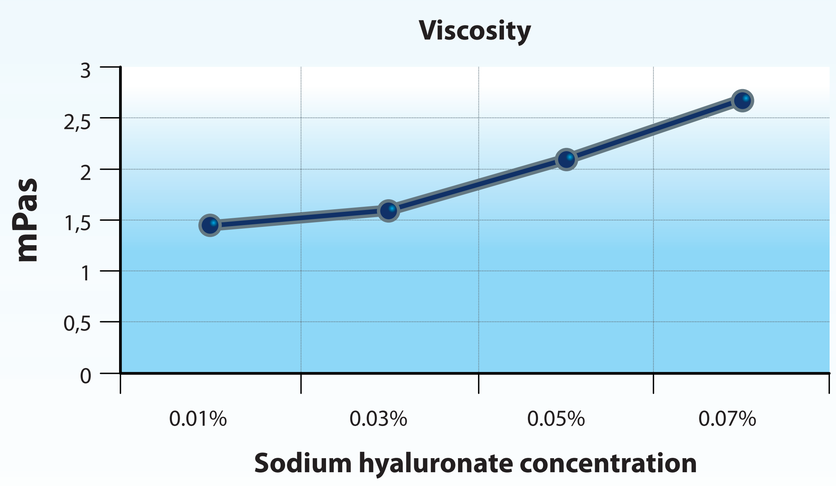
Four solutions of sodium hyaluronate were prepared (0.01%, 0.03%, 0.05% and 0.07%) and dynamic viscosity measured using a Höppler viscometer. Testing was performed on a 3K spray pump produced by AeroPump, Germany

Sodium hyaluronate	Viscosity (mPas)
0.01%	1,4504
0.03%	1,5876
0.05%	2,0956
0.07%	2,6821

RESULTS

There are significant changes in DSD values that follow increasement of viscosity. Increases were found in all three values, Dv(10), Dv(50) and Dv(90). Individual values Dv(10) and Dv(50) did not increase significantly in lower concentrations of sodium hyaluronate (between 0.01% and 0.03%), while the Dv(90) value showed an increase to a certain level. In addition, significant increases in all three droplet size distribution parameters were found between concentrations of 0.03% and 0.05%, and also 0.05% and 0.07%. Furthermore, there is also an indication that Dv(90) is more sensitive to changes of viscosity than Dv(10) and Dv(50).





CONCLUSION

There is a significant relationship between the rheological properties of a formulation and droplet size distribution, and as a consequence deposition of the drug product in the nasal cavity. A combination of determination of droplet size distribution by laser diffraction method and rheological measurements could aid researchers in understanding what kind of impact the changes in formulation could have on nasal spray functionality. These measurements are also useful in obtaining a combination of selected pump system and formulation that provides satisfactory deposition in the nasal cavity.

References:

[1] EMEA (2006), Guideline on pharmaceutical quality of inhalation and nasal products, QWP/49313/2005Corr

[2] Kippax P. et al.(2008), Enhancing the in vitro assessment of nasal sprays, Pharmaceutical Technology Europe

[3] Kippax P. et al.(2004), Manual versus automated

PROBLEM OF CHARACTERISING ICELAND MOSS DRY EXTRACT

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INTRODUCTION

The main problem in the production of Herbal Medicinal Products (HMP) is the high quality price – low product price [1]. Near-infrared and mid-infrared spectroscopy (NIRS: 10000-4000cm^{-1} ; MIRS: 4000-400cm^{-1}) are non-invasive, cheap spectroscopic tools enabling a fast qualitative characterisation of medicinal plants and their HMP [2].

The herb contains mucilage and bitter principles (lichenic acid, fumaroprotocetraric acid, protocetraric acid and cetraric acid) and other acids (lichesteric acid and usnic acid). Its constituents include approximately 50% water soluble polysaccharides, including lichenin, a linear cellulose-like polymer of β-D glucose and isolichenin, and a linear starch-like polymer of α-D glucose [3].

This is neither a standardised nor a quantified extract. It falls into the range of "other extracts", essentially defined by their production process (state of the herbal drug to be extracted: Iceland moss, solvent-water ratio 1:4, extraction conditions) and their specifications (containing Dextrin max. 30%). Most extracts belong to this type. All these specific parameters are indeed essential for the composition of the herbal preparation (active constituents, concomitants and impurities), and therefore for the quality, safety and efficacy of the product.

Cetraria islandica (L.)

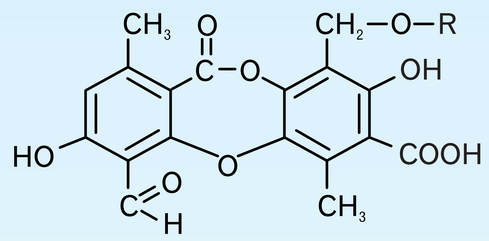
MATERIAL & METHOD

This paper presents the results of infrared spectroscopy measurements of the Iceland moss dry aqueous extract, *Cetraria islandica* (L.) *Acharius*, using the contemporary Attenuated Total Reflectance (ATR) – method with the Perkin Elmer Spectrum 100.

Fumaroprotocetraric acid:
R = -CO-CH=CH-COOH

Cetraric acid:
R = -C_2H_5

Protocetraric acid:
R = -H



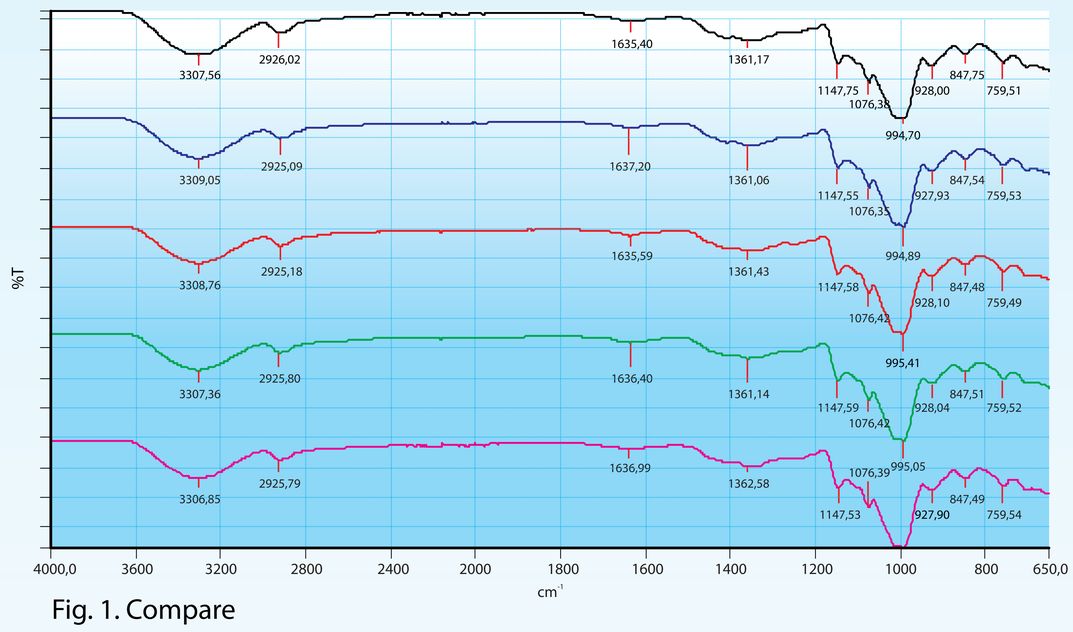


Fig. 1. Compare

Reference: Iceland moss dry extract 0319-09
Iceland moss dry extract 0445-09(IV) correlation 0.9957
Iceland moss dry extract 0445-09(II) correlation 0.9952
Iceland moss dry extract 0445-09(I) correlation 0.9951
Iceland moss dry extract 0445-09(III) correlation 0.9944

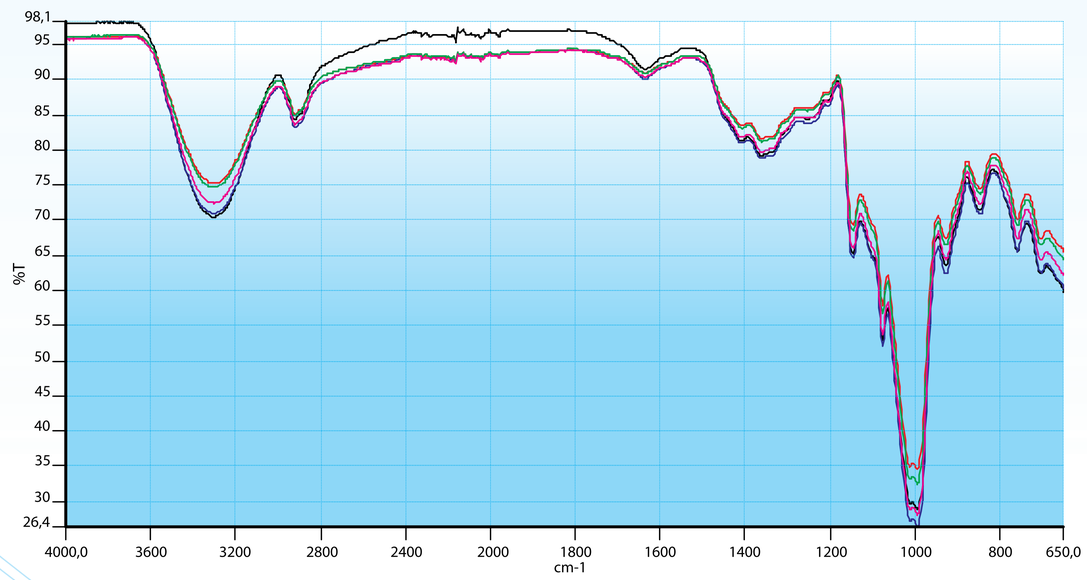


Fig. 2. Compare

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Iceland moss dry extract 0445-09(III) correlation 0.9944

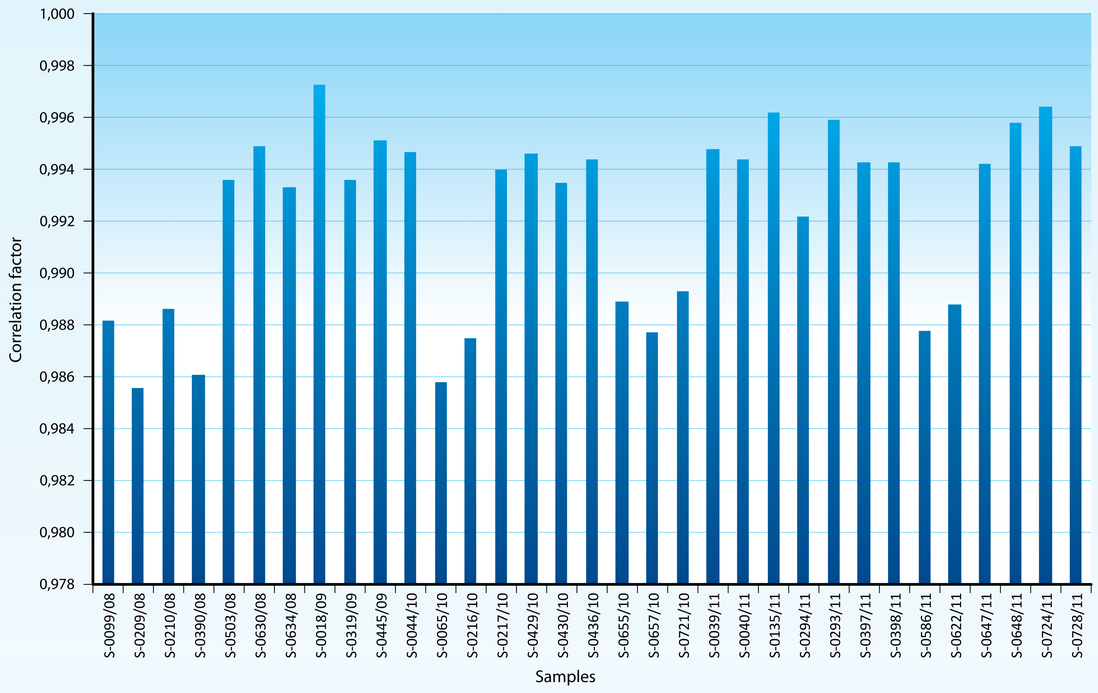

Fig. 3. Frequency correlation factor of IRS

RESULTS & DISCUSSION

It can often be very difficult to acquire samples of appropriate reference standards to make such comparable fingerprint chromatograms (Fig. 1.; 2.). This dry extract is improvable for TLC analysis, using the method in the herbal drugs monograph *Cetraria islandica* Ph Eur 7th ed. – identification of fumaroprotocetraric acid (analytical marker).

In the present contribution, the main advantages of the novel Infrared Spectroscopy (IRS) (4000-650cm^{-1}) quality control tool in medicinal plant analysis as a method of identification and quick qualitative characterisation is confirmed for Icelandic moss dry extract. The method was confirmed by a frequent correlation factor of IRS > 0.98 (0.9856 – 0.9973) on 33 samples over a period of three years (Fig. 3.). This raw material is used in the preparation of a syrup, in the food supplement category. From the pharmaceutical point of view, there are numerous possible components found in traces, which are thus difficult to identify. IRS is currently the principal tool for convenient plant analyses and preparations of medicinal plants.

References:

[1] Huck, CW. (2009) Innsbruck: Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Planta Med 75.

[2] Meier, B. (2003) Permanent Committee Manufacturing and Quality Control, GA-Workshop: NIRS, 51st GA annual congress, Kiel.

[3] Zovko, M. (2007) Characteristics, chemistry and use of lichens, Farm. Glas. 63: 227-243.

FOLIC ACID TABLET DISSOLUTION TESTING: HPLC AND UV METHOD VALIDATION AND COMPARISON

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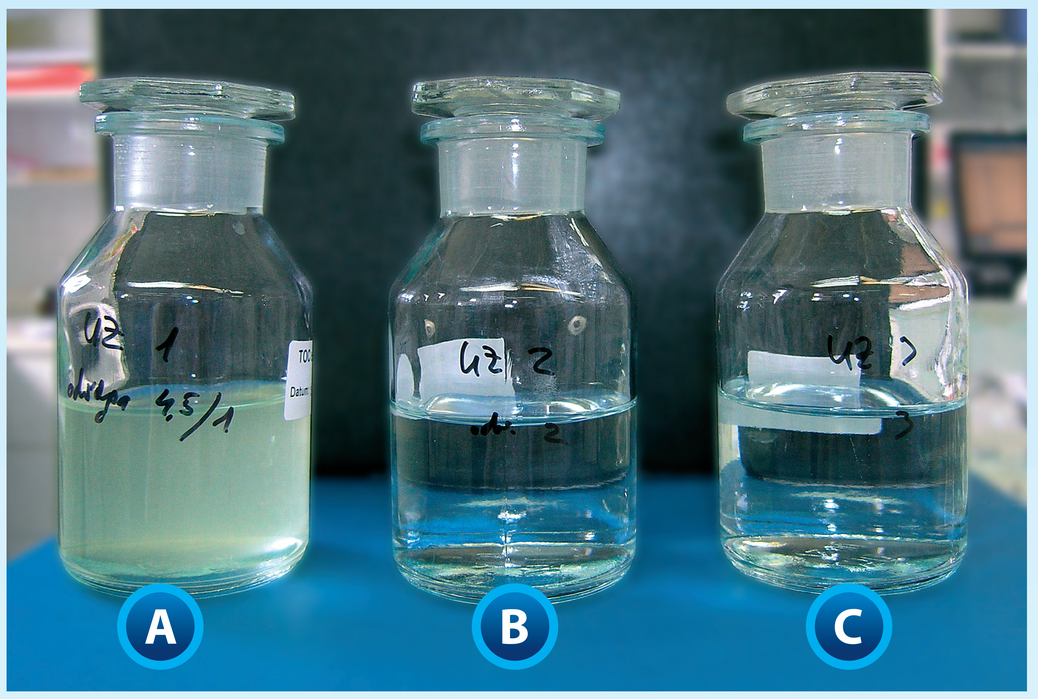
VARIAN VK 7010 DISSOLUTION APPARATUS

Picture 1: All folic acid dissolution tests were carried out by Varian VK 7010 dissolution apparatus

FOLIC ACID SOLUBILITY IN VARIOUS MEDIA



Picture 2: Folic acid solubility in water R (A), 0.05 M citrate buffer (B) and 0.05 M phosphate buffer (C).

INTRODUCTION

Present dissolution tests for folic acid tablets regulated by the USP and BP include HPLC methods for determining the % of folic acid dissolved in time.[1,2] Also, USP and BP dissolution tests include water as a dissolution medium (Table 1), stating that folic acid is practically insoluble in water.

The aim of this study was to develop and validate a method for determining the % of folic acid dissolved that would be simple and would include such dissolution medium in which folic acid is soluble and sink conditions can be achieved.

VARIOUS CONDITIONS FOR FOLIC ACID TABLET DISSOLUTION TESTING

	USP (HPLC) METHOD	NEW HPLC METHOD	UV METHOD
Medium	Water R	0,05 M phosphate buffer	0,05 M phosphate buffer
volume (ml)	500	900	900
Speed rate (rpm)	50	50	50
Temperature (Co)	37 ± 0.5	37 ± 0.5	37 ± 0.5
Time (min)	45	45	45

Table 1: Conditions for USP (HPLC), new HPLC and UV method for determining the % of folic acid released

MATERIALS AND METHODS

Folic acid solubility was tested in 4 different media (water, 0.1 M NaOH, 0.05 M citrate buffer and 0.05 M phosphate buffer). Different volumes of those 4 media were used for folic acid dissolution testing on Varian VK 7010 apparatus (Picture 1). The % of folic acid dissolved in time was determined by HPLC and UV method. HPLC analysis was carried out by Agilent 1100 series liquid chromatograph, using VWD detector; and UV analysis was carried out by Varian Cary 50 Bio spectrophotometer. Absorbances were measured at 282 nm.

HPLC and UV method were validated according to ICH guidelines for the validation of analytical procedures.

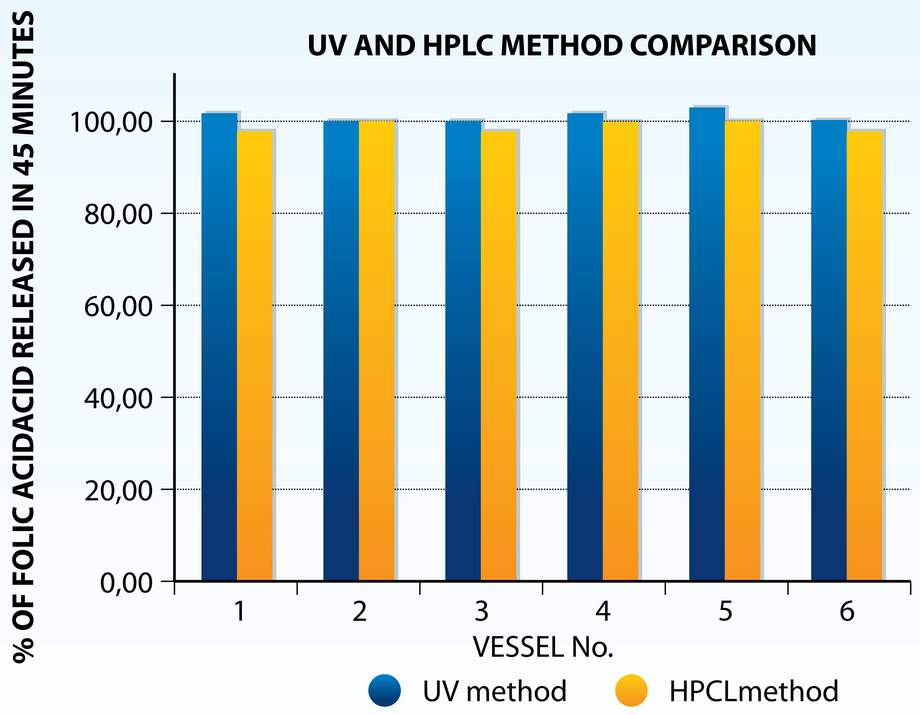
RESULTS

Folic acid was very easily soluble in 0.05 M citrate and 0.05 M phosphate buffer (Picture 2). Since citrate buffer is more agressive to HPLC column, it has been decided that 0.05 M phosphate buffer will be used for further testings.

Folic acid dissolution was better when 900 ml of medium was used. This volume ensures that sink conditions are achieved. Further testings were carried out in 900 ml of dissolution medium.

HPLC and UV analysis gained similar results: there was no significant difference between % of folic acid dissolved in time when analysis was carried out by HPLC apparatus in comparison with results obtained by UV analysis (Picture 3).

The linearity, accuracy, precision and robustness of the HPLC and UV method were tested and the satisfactory results gained proved that the proposed HPLC and UV methods for folic acid dissolution testing are accurate, precise and comparable.



Picture 3: Results for folic acid dissolved in time (%) when UV, i.e. HPLC method is used

CONCLUSION

Since there were no significant differences between HPLC and UV method, the latter was chosen for routine analysis, as well as for folic acid dissolution profile comparison, because of its simplicity and because it is less time-consumable.

References:

[1] Folic acid tablets official monograph. U. S. Pharmacopeia 34 2: 2911.

[2] Folic acid tablets monograph. British Pharmacopoeia 2007 3: 2603.

EVALUATION OF CELLULOSE POLYMERS IN OPHTHALMIC SOLUTIONS (EYE DROPS)

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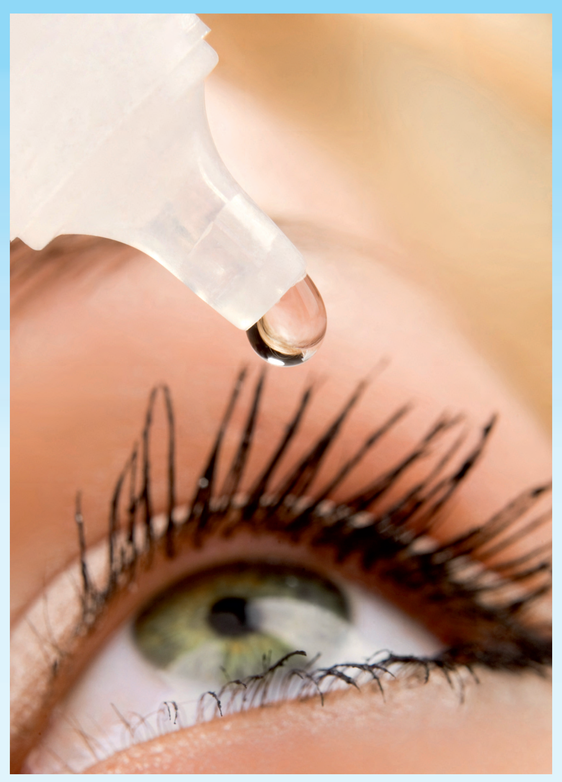
INTRODUCTION

Viscous solutions, based upon the addition of hydrocolloids to more simple aqueous solutions, are the most common formulations used[1].

HEC, partly substituted poly(hydroxyethyl) ether of cellulose is available in several grades that vary in viscosity and degree of substitution[2].

The rheological characteristics of HEC are implicated in the ocular retention of dosage form.



The aim of the study is to determine influence of hydroxyethyl cellulose (HEC) polymer viscosity grade types on the eye drops physical properties.

Requirements for ophtalmic solutions[3,4] during pharmaceutical development that have to be obtained:

- Sterility
- Isoosmolality
- Tolerance
- Patient comfort
- Ease of dispensing
- Antimicrobial preservation
- Ease of manufacture

SUBJECTS AND METHODS

Samples were prepared using different viscosity grade types and concentrations of HEC.

Table 1: Samples for analysis

Sample No.	HEC viscosity type (mPas)	HEC viscosity (mPas)	Conc. HEC (%)
1	1500-2500	1600	0.419
2	1500-2500	1600	0.467
3	1500-2500	1600	0.550
4	1500-2500	1750	0.419
5	1500-2500	1750	0.467
6	1500-2500	1750	0.550
7	1500-2500	1900	0.467
8	1500-2500	2150	0.467
9	3500-5500	3623	0.467
10	3500-5500	4500	0.467
11	3500-5500	5200	0.467

Table 2: Physical parameters tested

Physical parameters tested	Method	Instrument
Droplet size	In house	Density/specific gravity meter; Mettler Toledo DA-100-M
Viscosity	According to Ph.Eur.; 2.2.10	Viscometer Brookfield DV-II+PRO
Osmolality	According to Ph.Eur.; 2.2.35	Osmomat 030-D; Gonotec
Surface tension	In house	Tensiometer, Krüss, Easy dyne K20

RESULTS

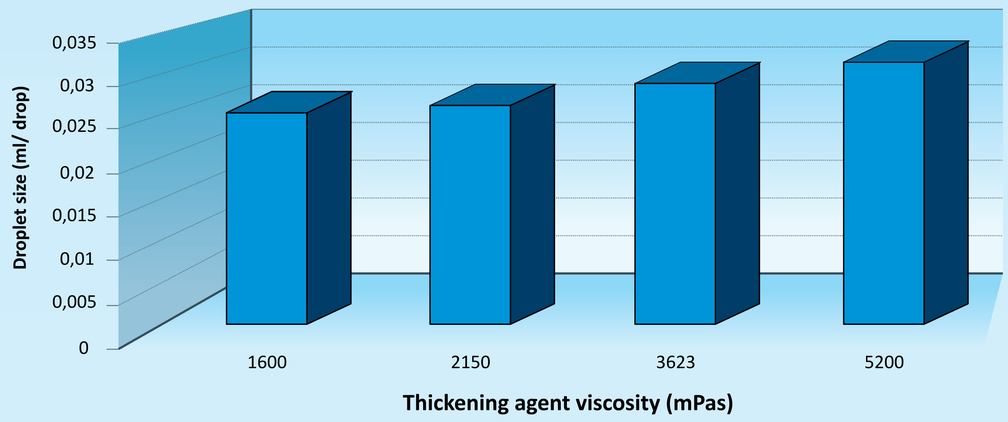


Figure 1: Variation of droplet size in relation to thickening agent viscosity

HEC concentration 0.467 %;
n=8 (numer of measurments per sample);
stdev: 0,0002, 0,0002, 0,0005, 0,0021.

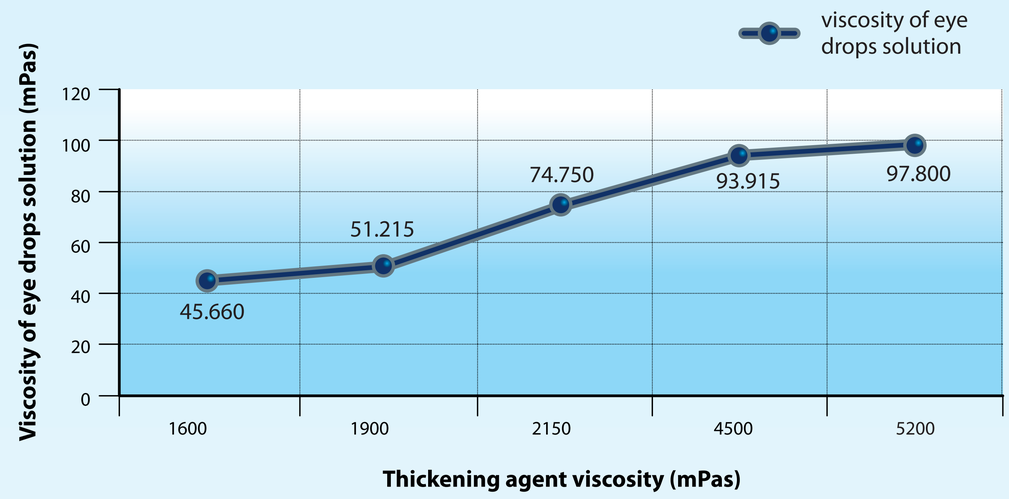


Figure 2: Viscosity of eye drops solution in relation to thickening agent viscosity

HEC concentration 0.467 %

Table 3: Viscosity of eye drops solution in relation to thickening agent concentration

HEC viscosity (mPas)	HEC conc. (%)	Viscosity of eye drops solution (mPas)
1600	0.419	34.42
1600	0.467	45.66
1600	0.550	82.71

Table 4: Osmolality and surface tension of eye drops solution in relation to thickening agent concentration

HEC viscosity (mPas)	HEC conc. (%)	Osmolality (osmol/kg)	Surface tension (mN/m)
1750	0.419	0.279	37.6
1750	0.467	0.279	37.7
1750	0.550	0.278	38.5

CONCLUSION

Droplet size depends on thickening agent viscosity. Comparing two HEC types, slighter variation of droplet size is noted in case of usage HEC grade type with viscosity range 1500-2500 mPas. Therefore, the viscosity range of HEC, its influence on droplet size and consequently on therapeutic dosage, should be considered. There is no impact of thickening agent viscosity and concentration on surface tension and osmolality. Viscosity of eye drops solution depends on thickening agent viscosity and concentration and it becomes higher with their increase. Selecting the adequate type of thickening agent, during preformulation study, is important to obtain formulation with desired physical properties.

References:

[1] Recent Advances in Ophthalmic Drug Delivery System; A.S.Mundada, J.G. Avari , S.P. Mehta, S.S. Pandit , A.T. Patil

[2] Handbook of Pharmaceutical Excipients, 6th edition

[3] Ocular preparations : The formulation Approach; Indu Pal Kaur, Meenakshi Kanwar ;

[4] Impact of Surface Tension in Pharmaceutical sciences; Anahita Fathi-Azarbayjani, Abolghasem Jouyban, Sui Yung Chan

DEVELOPMENT AND VALIDATION OF THE HPLC METHOD FOR ATENOLOL-RELATED SUBSTANCES IN TABLETS

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INTRODUCTION

Atenolol, (*RS*)-2-{4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl}acetamide, is a selective ß1 receptor antagonist, a drug belonging to the group of beta blocking agents (beta blockers). Atenolol in the form of tablets is used to treat hypertension (high blood pressure) and cardiac arrhythmias. It can also help to prevent angina pectoris (chest pain), and protect the heart in the early stages of treatment after myocardial infarction [1][2].

The HPLC method for atenolol-related substances in tablets has been described in the British Pharmacopoeia (2007) "Atenolol tablets"[3]. The stated method has not proved to be suitable in the determination of all known impurities of atenolol due to the overlapping of the peaks.

The aim of this study was, therefore, to develop and validate a precise, accurate and reproducible method for the determination of known atenolol-related substances in Atenolol tablets.

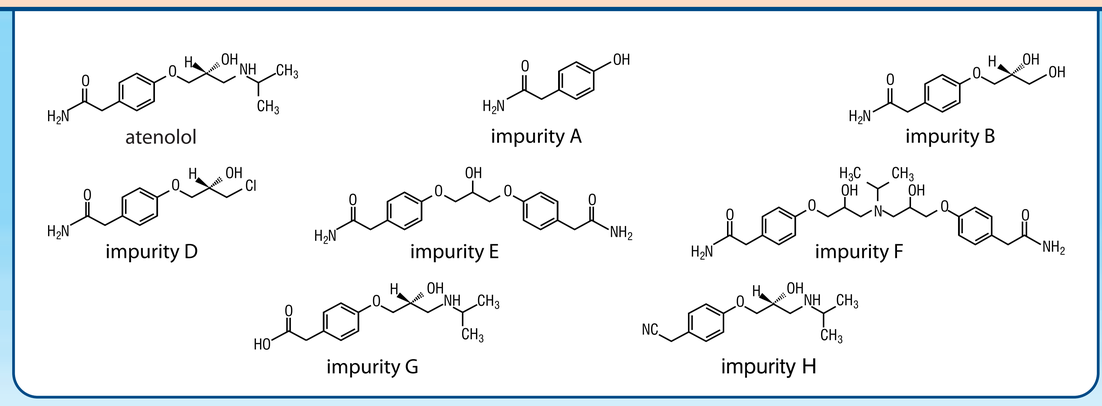

Figure 1. Molecular structures of Atenolol and impurities

METHOD DEVELOPMENT

After changing the chromatographic conditions (different columns, mobile phase and flow rate), the method has been optimised.
All peaks of impurities were well separated from each other and from the main atenolol peak.

Column	Lichrospher® 100 RP - 18 endcapped, 125 mm x 4.0 mm, 5µm
Column temperature	25°C
Mobile phase	dissolve 0.8 g of sodium octanesulfonate R and 0.4 g of tetrabutylammonium hydrogen sulfate R in 1 litre of a mixture of tetrahydrofuran - methanol - phosphate buffer pH 3.0 (2:18:80 V/V%)
Flow rate	0.6 ml/min
Injection volume	20 µl
Detection	226 nm (DAD)

Table 1. modified HPLC method

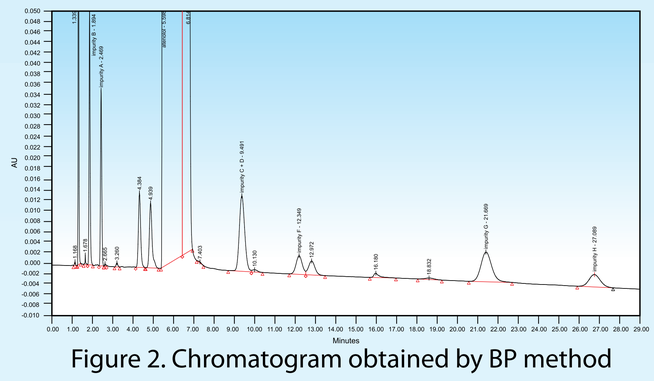

Figure 2. Chromatogram obtained by BP method

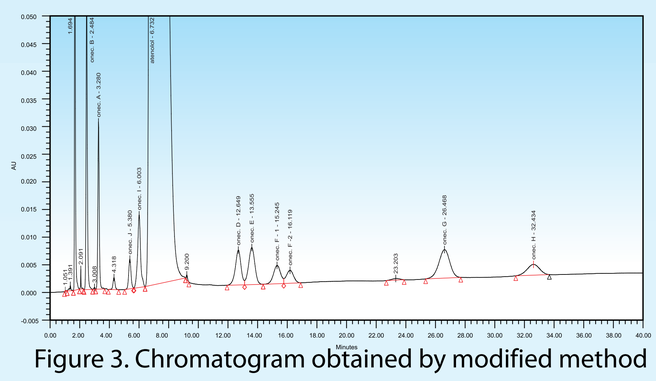

Figure 3. Chromatogram obtained by modified method

METHOD VALIDATION

The method was validated according to the ICH guidelines for validation of analytical procedures [4] with respect to selectivity, precision, accuracy, linearity, range, robustness, limit of detection and limit of quantification.

Accuracy, linearity, LOD and LOQ were tested for atenolol and impurities B, E, F and G because they are possible degradation products (according to DMF of manufacturer of API).

Selectivity

The method adequately separates all degradation products and placebo peaks from each other and from the main atenolol peak.

System suitability

Parameter	Requirement	Result
% RSD of area	max. 3.0%	0.62
Theoretical plates	min. 2000	5723
Resolution	min. 1.8	1.94

Table 2. Results for system suitybility test

Precision

Repeatability of the system

Repeatability of the system was determined by measuring reference solution six times. The calculated RSD of six injections is 0.37%.

Repeatability of the preparation

Repeatability of the preparation was determined by measuring individually prepared six sample solutions. RSD was calculated for impurities above limit of quantification.

	Peak area		
	Impurity A	**Impurity B**	**Impurity J**
Mean of six preparations (mV*s)	21034.4	49221.5	55350.5
RSD (%)	4.50	2.13	0.38

Table 3. Repeatability of sample solution preparation

Intermediate Precision

Compound name	Content (%)		RSD (%)	
	Analyst 1	**Analyst 2**	**Analyst 1**	**Analyst 2**
Impurity A	0.03	0.03	1.69	2.20
Impurity B	0.08	0.09	1.09	1.19
Impurity J	0.09	0.10	1.29	1.77
Total Impurities	0.21	0.22	1.15	1.56

Table 4. Intermediate precision

Accuracy

	Atenolol		Impurity B		Impurity E		Impurity F		Impurity G	
	Mean recovery	**RSD (%)**	**Mean recovery**	**RSD (%)**	**Mean recovery**	**RSD (%)**	**Mean recovery**	**RSD (%)**	**Mean recovery**	**RSD (%)**
0.1%	98.87	1.58	98.17	2.36	99.57%	0.50	94.83	1.76	93.67	2.44
0.5%	102.66	0.72	98.05	0.22	102.12	0.81	100.13	1.64	103.44	0.53
1.0%	100.36	0.91	94.68	0.28	102.04	0.23	98.64	0.73	102.39	0.42

Table 5. Accuracy

Linearity and range

Compound name	Equation of regression line	Coefficient of determination (R^2)	Range
Atenolol	y = 66245591.4x-255.2	0.9997	0.1 % - 1.0 % (0.001 mg/ml to 0.1 mg/ml)
Impurity B	y = 73941175.1x+5137.0	0.9997	
Impurity E	y = 97494000.3x-1637.0	1.0000	
Impurity F	y =72464138.8x -1911.8	0.9997	0.05 %-1.0 % (0.0005 mg/ml to 0.1 mg/ml)
Impurity G	y = 61693612.7x -3373.7	0.9998	

Table 6. Linearity and range of linearity

Robustness

Method condition	% RSD of area	Theoretical plates	Resolution
Mobile phase composition - 17% MeOH	0.53	5079	1.84
Mobile phase composition - 19% MeOH	0.47	5000	2.08
pH of buffer - 2.9	1.40	5054	2.01
pH of buffer - 3.1	0.68	5112	2.08
Flow rate - 0.5 ml/min	1.78	5566	2.25
Flow rate - 0.7 ml/min	1.82	4664	1.99
Column temperature - 23°C	0.96	4920	2.02
Column temperature - 27°C	0.97	5258	2.18

Table 7. Robustness of the method

Limit of detection and limit of quantification

	Atenolol	Impurity B	Impurity E	Impurity F	Impurity G
LOD	0.025%	0.025%	0.025%	0.050%	0.050%
LOQ	0.050%	0.050%	0.050%	0.100%	0.100%

Table 8. LOD and LOQ for atenolol and impurities

CONCLUSION

The new modified HPLC method for atenolol-related substances is more selective than the BP method.

The satisfactory results obtained from validation of the method proved the proposed method to be precise, accurate and reproducible, as well as stability indicating.

References:

[1] Medicines Complete, Martindale: The Complete Drug Reference, Atenolol, available at http://www.medicinescomplete.com/mc/martindale/current/index.htm.

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