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Short Communication

VALIDATION OF THE QUALITATIVE DETERMINATION OF HPLC METODS FOR SUCROSE AND PEG-2000-DSPE IN A LIPOSOMAL FORM OF THE PHOTOSENSITIZER LIPOPHTHALOCYAN

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ABSTRACT

Objective: This study was undertaken with the aim of the validate the simple isocratic metods high-performance liquid chromatographic (HPLC) for the estimation of PEG-2000-DSPE and sucrose in liposomal medicinal formulations of the phthalocyanine photosensitizer Lipophthalocyan.

Methods: HPLC quantification was carried out by using of YMC-Pack Polyamine II column. The mobile phase (for sucrose: acetonitrile: water: ethyl acetate in the ratio of 450: 200: 20; for PEG-2000-DSPE: water in the ratio 10: 90) was pumped at a flow rate of 1 ml/min. Following the guidelines of the International Conference on Harmonization (ICH), the methods was validated for various analytical parameters like specificity, linearity, detection limit, quantitative limit, correctness, and accuracy.

Results: The obtained results of the analysis were validated statistically. The correlation coefficient for the linearity was 0.999292, for sucrose, and 0.997650 for PEG-2000-DSPE. The methods can be assessed as correct, as the results obtained are close to the true value and the confidence interval for both methods include 100%. The coefficients of variation in both methods in determining the accuracy were less than 3%.

Conclusion: The proposed HPLC methods were validated according to the ICH guidelines and results and statistical parameters demonstrated that the developed methods are sensitive, precise, reliable and simple for the estimation of PEG-2000-DSPE and sucrose in Lipophthalocyan.

Keywords: HPLC, Sucrose, PEG-2000-DSPE, Lipophthalocyan, Validation

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The development and production of innovative, safe and highly effective medicines with low toxicity is a priority goal for the pharmaceutical industry. One of the ways in which the effectiveness of medicinal therapies can be increased is through the application of nanotechnologies, specifically the creation of nanotechnology-based delivery systems. Staff at the N. N. Blokhin National Medical Research Center of Oncology developed Lipophthalocyan, an injectable liposomal formulations of an antineoplastic hydrophobic photosensitizer, the active component of which is tetra-3-phenylthiophthalocyanine aluminum hydroxide. The advantages of this medicine include its high degree of selectivity in targeting the nidus of a tumor, its low toxicity for the system and its high level of effectiveness, as confirmed by in vivo and in vitro testing [1]. The proposed drug has considerable potential for use in photodynamic therapy for surface and internal neoplasms, particularly cancers of the skin and lower lip and tumors of the tunica mucosa of the mouth and tongue.

For the quantitative determination of substances in liposomes, HPLC, HPTLC, capillary electrophoresis and spectrophotometry are used [2-5]. Selection of one method or another depends on the specific task facing the investigator. The content of tetra-3-phenyl-thiophthalocyanine aluminum hydroxide (the active component) is determined through spectrophotometry. Sucrose, phosphatidylcholine and cholesterol can be measured using TLC with chromatodensitometry [6]. However, because of the low level of PEG-2000-DSPE and the high level of sucrose in the Lipophthalocyan, the HPLC method was selected for quantitative analysis. When developing new methodologies it is essential to identify any defects at an early stage through the use of validation, in order to obtain reliable and accurate results [7, 8]. The purpose of this work develop and validation simple, sensitive, specific, accurate HPLC methods for the estimation of sucrose and PEG-2000-DSPE in the liposomal antineoplastic photosensitizer Lipophthalocyan.

Lipophthalocyan (N. N. Blokhin National Medical Research Center of Oncology, Russia), sucrose for HPLC (CAS 57-50-1, Sigma-Aldrich), PEG-2000-DSPE (CAS 474922-22-0, Lipoid, Germany), ethanol 95% (ZAO Bryntsalov-A Ferein, Russia), acetonitrile for HPLC (CAS 75-05-8, Sigma-Aldrich), ethyl acetate for HPLC (CAS 141-78-6, Sigma-Aldrich), HPLC on a chromatograph of Aliance with a refractometric detector of Waters 2414 (Waters, USA), column of YMC-Pack Polyamine II 5 μ, 12 nm, 250×4.6 mm (YMC, USA), Transsonic ultrasound bath (Elma, Germany).

For the determination of sucrose, the contents of a vial of the medicinal formulation of Lipophthalocyan were dissolved in 6 ml of water, shaken thoroughly, and left in an ultrasound bath for 30-40 min for complete dissolution of sucrose. The resulting solution was diluted 10-fold: 10 ml of solution was transferred to a 1 ml pycnometer, made up to the mark with water, and mixed thoroughly. A 1 ml aliquot of the resulting solution was placed in an Eppendorf microcentrifuge tube and centrifuged at 18.000 rpm for 5 min. A 0.9 ml aliquot of the resulting solution was transferred to a vial and analyzed by HPLC.

For the determination of PEG-2000-DSPE the contents of a vial of the medicinal formulation of Lipophthalocyan were dissolved in 5.8 ml of ethanol, shaken thoroughly, and left in an ultrasound bath for 30-40 min for complete dissolution of PEG-2000-DSPE. A 1 ml aliquot of the resulting solution was placed in an Eppendorf microcentrifuge tube and centrifuged at 18.000 rpm for 5 min. A 0.9 ml aliquot of the resulting solution was transferred to a vial and analyzed by HPLC.

HPLC quantification was carried out by using of YMC-Pack Polyamine II column with Rf-detectorom. Column temperature 50 ° C. The mobile phase (for sucrose: acetonitrile: water: ethyl acetate in the ratio of 450: 200: 20; for PEG-2000-DSPE: water in the ratio 10: 90) was pumped at a flow rate of 1 ml/min. Analysis time for sucrose was 10 min and for PEG-2000-DSPE 20 min.

Data reception and processing were performed using the Empower Pro computer system. The proposed HPLC method was confirmed as per ICH [8].

Methods were evaluated according to the following criteria: 1) specificity-placebo peaks must not overlap the active substance

peak; 2) detection limit (DL)-the signal: noise ratio must not be less than 5/1; 3) quantitative limit (QL)-the signal: noise ratio must not be less than 10/1; 4) linearity-the correlation coefficient must be R>0,99; 5) correctness-the confidence interval was included 100% of measurements; 6) accuracy-the coefficient of variation of peak areas must be less than 10.

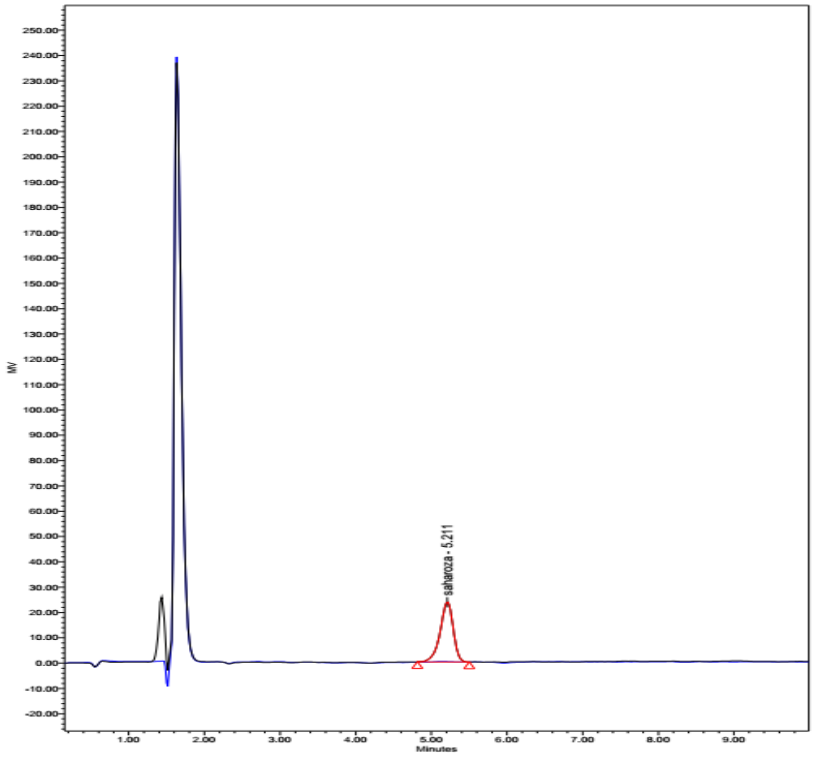

Fig. 1: Overlay chromatograms of a sucrose standard at a working concentration of 10 mg/ml and placebo

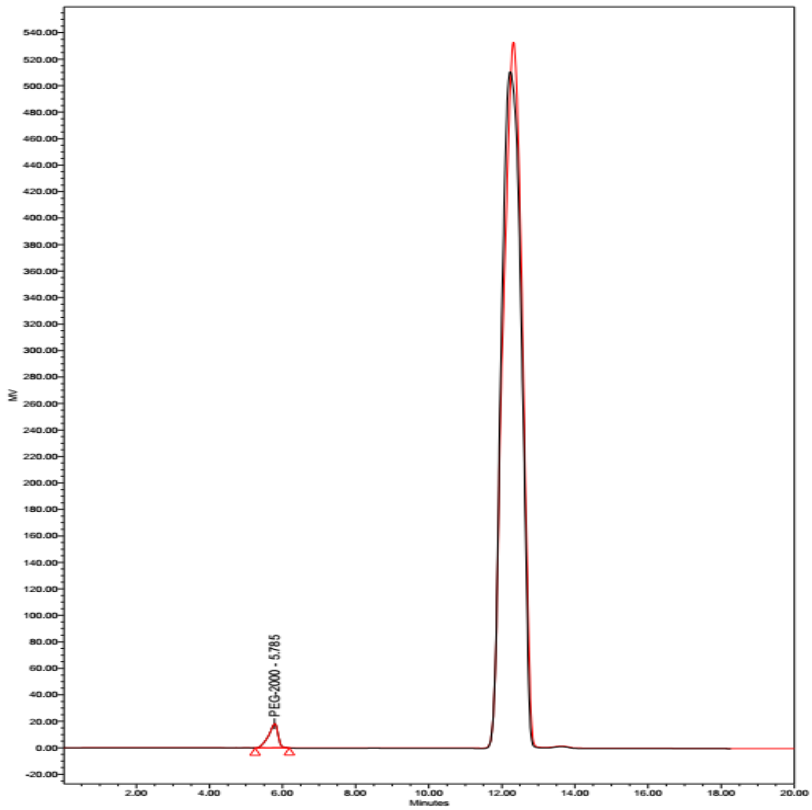

Fig. 2: Overlay chromatograms of a PEG-2000 standard at a working concentration of 0.5 mg/ml and placebo

Specificity was determined using placebo chromatograms (i.e., the medicinal formulation of Lipophthalocyan without the substance being assayed) and standards for sucrose (fig. 1) and PEG-2000-DSPE (fig. 2). As shown in fig. 1-2, placebo did not produce sucrose or PEG-2000-DSPE peaks. Accordingly, it can be concluded that the method developed is said to be specific [8].

To determine the detection limit (DL) and Quantitative limit (QL) of sucrose, solutions of 0.05%, 0.1%, 0.2%, 0.3%, 0.5% and 1% of the nominal working concentration (10 mg/ml) were prepared. The DL of the sucrose was defined as a concentration giving a signal 5 times higher than the background noise level, and it was established as 0.05% of the nominal working concentration of working standard sucrose (0.5 mg/ml). The QL was defined as a concentration giving a signal 10 times higher than the background noise level, and it was established as 0.1% of the nominal working concentration of working standard sucrose (1 mg/ml).

To determine the detection limit (DL) and Quantitative limit (QL) of PEG-2000-DSPE, solutions of 0.1%, 0.2%, 0.3%, 0.5% and 1% of the nominal working concentration (0,5 mg/ml) were prepared. The DL of PEG-2000-DSPE was defined as a concentration giving a signal 5 times higher than the background noise level, and it was established as 0.1% of the nominal working concentration of working standard PEG-2000-DSPE (0.05 mg/ml). The QL was defined as a concentration giving a signal 10 times higher than the background noise level, and it was established as 0.2% of the nominal working concentration of working standard sucrose (0.1 mg/ml). The results of determining the DL and QL indicate the high sensitivity of the methods and meet the requirements of the ICH [8].

In order to confirm linearity, calibration curves correlating the concentrations with the peak area were plotted (fig. 3-4) and a correlation coefficient R was calculated. This was 0.999292, for sucrose and 0.997650 for PEG-2000-DSPE. The closeness of the value of the correlation coefficients to 1 indicates a fairly close linear correlation between the concentrations and the peak area [7, 8].

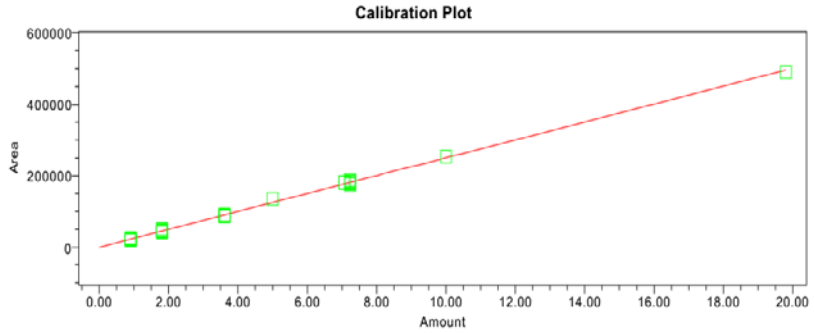

Fig. 3: The linearity graph of sucrose in the range from 1 to 20 mg/ml

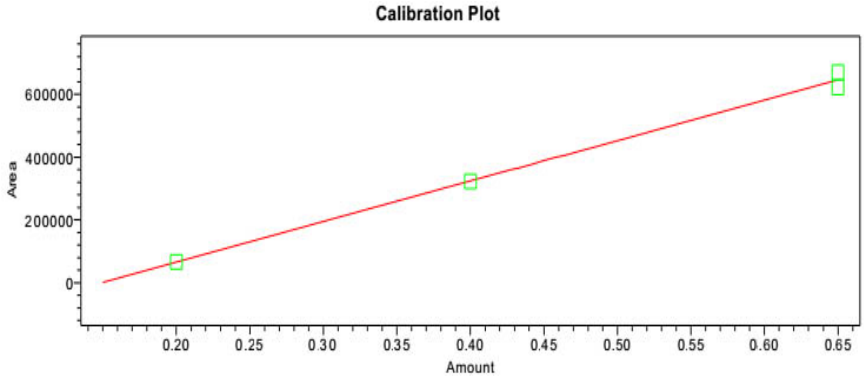

Fig. 4: The linearity graph of PEG-2000-DSPE in the range from 0.2 to 0.65 mg/ml

In order to demonstrate correctness, standard solution samples were prepared, in which the quantities of the components were known. Correctness was calculated as the percentage of consistency between the observed and given quantitative values for the contents of the samples. On the basis of the results of the research, shown in table 1, the methods can be assessed as correct, as the results obtained are close to the true value and the confidence interval for both methods is 100% [8].

Table 1: Results of the assessment of the correctness of the methods

Sucrose			PEG-2000-DSPE		
Added concentration, mg/ml	**Found, mg/ml**	**Response, %**	**Added concentration, mg/ml**	**Found, mg/ml**	**Response, %**
1.95	1.953	101.2	0.2	0.197	98.5
	1.947	98.8		0.215	107.5
	1.951	100.4		0.201	100.5
3.5	3.498	99.6	0.4	0.408	102.0
	3.494	98.8		0.424	106.0
	3.519	103.6		0.398	99.5
7.7	7.747	99.6	0.65	0.647	99.5
	7.752	100.3		0.659	101.4
	7.751	100.1		0.652	100.3
confidence interval	100.28±1.17		confidence interval	101.69±2.36	

Table 2: Results of determination of accuracy of sucrose in the lipophthalocyan (part. 020916)

No.	The sucrose content of the Lipophthalocyan			
	Analyst 1		Analyst 2	
	d 1	d 2	d 1	d 2
1	570	594	582	612
2	606	606	588	582
3	588	618	618	588
4	576	576	612	582
5	594	612	576	624
6	612	618	624	606
Coefficient of variation	2.19	1.78	2.25	1.47

Table 3: Results of determination of accuracy of PEG-2000-DSPE in the lipophthalocyan (part. 020916)

No.	The sucrose content of the lipophthalocyan			
	Analyst 1		Analyst 2	
	d 1	d 2	d 1	d 2
1	258	249	244	255
2	245	253	256	249
3	247	252	255	248
4	252	247	247	251
5	253	246	246	254
6	244	254	252	253
coefficient of variation	1.19	0.78	1.25	1.47

To determine accuracy, the results of the analysis performed in identical conditions (i.e. using the same equipment) on different days and by different researchers were compared.

The coefficient of variation when determining accuracy was 1.47-2.25% for sucrose in the Lipophthalocyan (table 2) and 0.78-1.47% for PEG-2000-DSPE in the Lipophthalocyan (table 3), which indicate the methods was accurate [8].

In accordance with the requirements of the ICH, validation was performed in respect of HPLC methods for the determination of the quantities of sucrose and PEG-2000-DSPE in the liposomal drug Lipophthalocyan. These methods proved to be sufficiently replicable, highly sensitive, and simple to perform, and had short chromatography times. They can therefore be used for regular qualitative and quantitative analysis of sucrose and PEG-2000-DSPE levels in Lipophthalocyan.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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