

ANALYSIS

DEVELOPMENT AND VALIDATION OF THE GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF ORGANIC VOLATILE IMPURITIES IN TRAVOPROST AND ITS STARTING MATERIAL

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Abstract: The methods for controlling organic volatile impurities, including residual solvents and reagents, in the travoprost active pharmaceutical ingredient, has been reported. Monitoring the chemical purity of the starting material – TV-38A ((R)-(-)-2-(tert-Butyldimethylsilyloxy)-3-(3-trifluoromethylphenoxy)propan-1-ol) – is critical, as it may directly affect the impurity profile and the quality of the final product. The proposed methods have been developed using gas chromatography as well as gas chromatography with headspace injection and they have been validated according to the requirements of the ICH (International Conference of Harmonization) Q2R1 validation guidelines and the Q3C guideline for residual solvents.

Keywords: GC method, GC-HS method, validation, travoprost, residual solvents, organic volatile impurities

Travoprost (5Z,13E)-(9S,11R,15R)-9,11,15-trihydroxy-16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-5,13-prostadienoic acid, isopropyl ester, is a new active substance, a prostaglandin analogue ester prodrug of the active moiety (+)-fluprostenol, intended to reduce intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension (1, 2). Our new method for travoprost synthesis has been published recently (3, 4). A detailed description of the manufacturing process was described including the reaction conditions, quantities of materials and yields, as well as the process and chemical flowchart. The main impurities identified are the C5-C6 trans isomer, and the travoprost free acid, both synthetic by-products. The impurity limits in the specifications were justified by toxicological studies.

To continue our studies on the active pharmaceutical ingredient (API) – travoprost, gas chromatography methods for the determination of residual solvents, formerly called organic volatile impurities (OVI), have been elaborated and verified in a validated analytical procedure. It was also critical to monitor the chemical purity of the starting material – TV-38A ((R)-(-)-2-(tert-Butyldimethylsilyloxy)-

3-(3-trifluoromethylphenoxy)propan-1-ol), because it could have a direct effect on the impurity profile and the quality of the final product. Acceptable levels of many residual solvents and limits, mainly based on toxicity, are included in the regulatory guidance documents, in particular in the Q3C guideline issued by the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) (5). Taking into account their relative toxicity, these solvents should be monitored in the API at various levels, ranging from 2 ppm (2 µg/g drug substance) for benzene (Class 1) to 0.5% for the Class 3 solvents.

During the synthesis of travoprost, the following solvents and volatile reagents were used: methanol, acetone, 2-propanol, acetonitrile, dichloromethane, ethyl acetate, tetrahydrofuran, 2-iodopropane, ethanol, methyl *t*-butyl ether, hexane, toluene, pyridine, N,N-dimethylformamide, and TV-38A. It was necessary to control residual benzene in travoprost because benzene is a potential contaminant of acetone and toluene. Consequently, the analytical methods used to monitor these solvents and reagents needed to cover a large range of boiling

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points and polarities. This paper describes the development and validation of two methods: gas chromatography with headspace injection (GC-HS) as well as gas chromatography with direct injection (GC) for analyzing organic volatile impurities in travoprost and its starting material. Our analytical studies include the following validated methods: method I – a GC-HS method for the determination of the specified residual solvents (methanol, acetone, 2-propanol, acetonitrile, dichloromethane, ethyl acetate, tetrahydrofuran, and 2-iodopropane); method II – a GC-HS method for the determination of the unspecified residual solvents (ethanol, methyl *t*-butyl ether, hexane, and toluene); method III – a GC-HS method for the determination of benzene; method IV – a GC method for the determination of pyridine and *N,N*-dimethylformamide; method V – a GC method used for the purity control of the starting material TV-38A (normalization method procedure).

In literature, there are many LC methods for the determination of travoprost. High-performance liquid chromatography methods were developed to determine travoprost in a pharmaceutical formulation (6, 7) or in eyelash enhancing cosmetic serums (8). Our analytical methods are the first GC methods ever to be reported for the organic volatile impurity determination in the travoprost active substance and its starting material. The official monographs: USP (United States Pharmacopeia Convention) and EP (European Pharmacopoeia) mention no documents related to travoprost. All revealed validation results meet the requirements of the ICH Q2R1 validation guidelines (9) and the Q3C guideline for residual solvents (5). The described GC methods may be applicable for the impurity analysis of other prostaglandins.

EXPERIMENTAL

Chemicals and reagents

The active substance travoprost and the starting material TV-38A were synthesized in Pharmaceutical Research Institute (Warsaw, Poland). The solvents and diluents were purchased from commercial suppliers: 2-iodopropane from Aldrich (Germany), methanol, ethanol, acetone, 2-propanol, acetonitrile (ACN), dichloromethane (DCM), hexane, ethyl acetate, tetrahydrofuran (THF), toluene, *N,N*-dimethylacetamide (DMA) from POCh (Poland), methyl *t*-butyl ether (MTBE), pyridine, *N,N*-dimethylformamide (DMF) from Merck (Germany), benzene from Fluka (Germany).

Equipment

Methods I, II and III were performed on a Perkin Elmer CLARUS 500 gas chromatograph with a flame ionization detector interfaced with a Perkin Elmer headspace TURBOMATRIX 40 autosampler. Methods IV and V were conducted on a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector, a split injector and a Shimadzu AOC-20i autosampler. A DB-624 column (phase composition: 6% cyanopropylphenyl – 64% dimethylpolysiloxane) film thickness 1.8 μm , 60 m long and 0.32 mm ID, from Agilent Technologies, was used in methods I, II, III and IV. In method V chromatographic separation was performed on a DB-5 column (95% dimethyl-5% diphenyl polysiloxane), 30 m long, 0.32 mm ID, 1 μm film thickness, from Agilent Technologies.

Method description

Method I – determination of the specified residual solvents

Control of residual methanol, acetone, 2-propanol, acetonitrile, dichloromethane, ethyl acetate, tetrahydrofuran and 2-iodopropane in travoprost by method I is a quantitative test procedure because these compounds were used in the last step of the synthesis.

Chromatographic conditions

The following oven temperature program was used: the initial temperature of 45°C was held for 9 minutes and raised at the rate of 5°C/min to reach 60°C, then increased at 10°C/min to 140°C, next increased at 40°C/min to 240°C and held at this level for 5 min. The injection port temperature was 240°C and the detector temperature was 260°C. Nitrogen was used as the carrier gas at 100 kPa, split 5 : 1, attenuation-5. The vial oven temperature was set at 100°C for 30 min. The needle temperature was 110°C, the transfer line was 120°C, inject: 0.07 min.

Sample and standard solution preparation

The sample solution was prepared by dissolving an appropriate amount of travoprost in DMA with the addition of H_2O (5 : 1) to obtain a concentration of 6%. The standard solution was prepared by dissolving appropriate amounts of determined residual solvents in DMA with the addition of H_2O and then dilution to reach 3000 $\mu\text{g/mL}$ of methanol, 5000 $\mu\text{g/mL}$ of acetone, 5000 $\mu\text{g/mL}$ of 2-propanol, 410 $\mu\text{g/mL}$ of acetonitrile, 600 $\mu\text{g/mL}$ of dichloromethane, 5000 $\mu\text{g/mL}$ of ethyl acetate, 720 $\mu\text{g/mL}$ of tetrahydrofuran, 360 $\mu\text{g/mL}$ of 2-iodopropane with respect to the sample preparation (100% of the per-

missible limit). The blank solution was formed from DMA and H₂O (5 : 1).

Method II – determination of the unspecified residual solvents

Control of residual ethanol, methyl *t*-butyl ether, hexane and toluene in travoprost by method II is a limit test procedure (below 10% of the permissible limits for the solvents) because these solvents were used prior to the last step of the synthesis and were not detected in the tested batches of the substance.

Chromatographic conditions

The following oven temperature program was used: the initial temperature of 45°C was held for 9 minutes and raised at the rate of 5°C/min to reach 60°C, then increased at 10°C/min to 140°C, next increased at 40°C/min to 240°C and held at this level for 5 min. The injection port temperature was 240°C and the detector temperature was 260°C. Nitrogen was used as the carrier gas at 100 kPa, split 5:1, attenuation-5. The vial oven temperature was set at 100°C for 30 min. The needle temperature was 110°C, the transfer line was 120°C, inject: 0.07 min.

Sample and standard solution preparation

The sample solution was prepared by dissolving an appropriate amount of travoprost in DMA with the addition of H₂O (5 : 1) to obtain a concentration of 6%. The standard solutions were prepared by dissolving appropriate amounts of determined residual solvents in DMA with the addition of H₂O and then dilution to reach 500 µg/mL of ethanol, 500 µg/mL of methyl *t*-butyl ether, 29 µg/mL of hexane, 89 µg/mL of toluene with respect to the sample preparation (10% of the permissible limit). The blank solution was formed from DMA and H₂O (5 : 1).

Method III – determination of benzene

Control of residual benzene in travoprost by method III is a limit test procedure (below 30% of the permissible limit for benzene) because benzene is a potential contaminant of acetone, toluene and was not detected in the tested batches.

Chromatographic conditions

The oven temperature program was as follows: the initial temperature of 35°C was raised at the rate of 2°C/min to 70°C and it was maintained for 2 min before it was raised again at the rate of 40°C/min to 240°C and maintained for 6 min. The injection port

temperature was 240°C and the detector temperature was 260°C. Nitrogen was used as the carrier gas at 100 kPa, split 3 : 1, attenuation-5. The vial oven temperature was set at 95°C for 30 min. The needle temperature was 110°C, the transfer line was 120°C, inject: 0.07 min.

Sample and standard solution preparation

The sample solution was prepared by dissolving an appropriate amount of travoprost in DMA with the addition of H₂O (1 : 1) to obtain a concentration of 10%. The standard solutions were prepared by dissolving appropriate amounts of benzene in DMA with the addition of H₂O and dilution to reach 2 µg/mL with respect to the sample preparation (100% of the permissible limit) and 0.6 µg/mL with respect to the sample preparation (30% of the permissible limit). The blank solution was formed from DMA and H₂O (1 : 1).

Method IV – determination of pyridine and DMF

Control of residual pyridine and DMF in travoprost by method IV is a limit test procedure (below 10% of the permissible limits for the solvents) because these solvents were used prior to the last step in the synthesis and were not detected in the tested batches of the substance.

Chromatographic conditions

The oven temperature program was as follows: the initial temperature of 60°C was raised at the rate of 2°C/min to 60°C, then increased at 40°C/min to 240°C and held at this level for 5 min. The injection port temperature was 240°C and the detector temperature was 260°C. Nitrogen was used as the carrier gas at 100 kPa, split 5:1, 1 µL of the solution was injected into the gas chromatograph.

Sample and standard solution preparation

The sample solution was prepared by dissolving an appropriate amount of travoprost in ACN to obtain a concentration of 4%. The standard solutions were prepared by dissolving appropriate amounts of pyridine and DMF in ACN and then dilution to reach 20 µg/mL of pyridine and 88 µg/mL of DMF with respect to the sample preparation (10% of the permissible limit). The blank contained only ACN.

Method V – purity control of the starting material TV-38A

The purity of TV-38A – starting material in the travoprost synthesis – is controlled by method V with the area normalization method for calculation.

Chromatographic conditions and sample solution preparation

The oven temperature program was as follows: the initial temperature of 60°C was held for 4 min, raised at the rate of 10°C/min to 280°C and held at this level for 14 min. The injection port temperature was 180°C and the detector temperature was 280°C. Nitrogen was used as the carrier gas at 60 kPa, split 20 : 1, 0.5 µL of the sample solution was injected into the gas chromatograph. The sample solution was prepared by dissolving 50 µL of the examined substance in 2-propanol in a 1.0 mL volumetric flask (the nominal concentration in the sample solution).

RESULTS AND DISCUSSION

Method development

A column coated with a thick film of a medium polar stationary phase (DB-624) was selected for methods I-IV and a nonpolar stationary phase (DB-5) was selected for method V. Good separations were obtained on these columns in about 30 min of the analysis time. The influence of the solvent used to dissolve travoprost was evaluated. The LOD (and the slope of the calibration curve) depends on the solvent used. The LOD was lower (more sensitive), if the static headspace was used in DMA/water versus pure DMA. In general, equally good quantitative data in terms of linearity and repeatability were also obtained when using DMA/water. The presence of a relatively high concentration of travoprost in a sample solution (60 mg/mL) might influence the absolute response of the solutes. In order to evaluate the potential of this method, identical GC-HS analyses were performed on 12 sample solutions (triplicate independent preparations of four solutions – the sample spiked with the analytes at about 10%, 50%, 100%, 120% of the specification limit). The response for the samples spiked with the solvents was between 80 and 120 percent. This recovery is well within the limits generally accepted for the trace impurity analysis.

Method validation

The validation of method I (quantitative test procedure) includes the examination of specificity and selectivity, linearity, accuracy, repeatability, intermediate precision, system suitability, robustness as well as the quantitation and detection limits. The validation of methods II, III and IV (limit test procedures) includes only the examination of specificity, detection limit and, additionally, system suitability. The validation of method V (purity control)

includes the examination of the specificity, linearity, precision, the range in which the normalization method is correct and the detection limit.

The following acceptance criteria were established for methods I, II, III and IV:

- Specificity: resolution (R_s) = 1.5
- Linearity: correlation coefficient $R^2 \geq 0.990$, $y = ax + b$, parameters of Student's t-test: $t_a \geq t_{cr}$; $|t_b| < t_{cr}$; $|t_r| > t_{cr}$; $t_{cr} = 2.78$ ($n = 6$) ($\alpha = 0.05$, $n - 2$), parameter of Shapiro-Wilk test: $W \geq W_{cr}$; $W_{cr} = 0.788$ ($n = 6$) ($\alpha = 0.05$)
- Range: 10% ÷ 120% of the specification limit (the interval between the upper and lower concentration of the analytes in the sample for which the method is linear, accurate and precise)
- Accuracy: relative standard deviation (RSD) ≤ 15%, recovery: 80% ÷ 120%
- Precision (Repeatability and Intermediate precision): RSD = 15%, Snedecor's F-test: $F \leq F_{cr}$; $F_{cr} = 5.05$ ($n = 6$) ($\alpha = 0.05$, $f_1 = n_1 - 1$, $f_2 = n_2 - 1$)
- System suitability: RSD ≤ 1% (retention time – t_R), RSD ≤ 10% (peak area)
- Robustness: $R_s = 1.5$
- Detection limit (based on the signal-to-noise ratio): S/N = 3
- Quantitation limit (based on the signal-to-noise ratio): S/N = 10

The following acceptance criteria were established for method V:

- Specificity: $R_s \geq 1.5$
- Linearity: correlation coefficient $R^2 \geq 0.990$, $y = ax + b$, parameters of Student's t-test: $t_a \geq t_{cr}$; $|t_b| < t_{cr}$; $|t_r| > t_{cr}$; $t_{cr} = 3.18$ ($n = 5$) ($\alpha = 0.05$, $n - 2$)
- Precision: RSD ≤ 1% (t_R), RSD ≤ 5% (area), RSD ≤ 1% for the main compound and RSD ≤ 5% for the impurities (assay%)
- Range of the area normalization method: 80% ÷ 120 % of the nominal concentration in the sample solution or larger, RSD ≤ 1% for the main compound and RSD ≤ 5% for the impurities
- Detection limit (based on the signal-to-noise ratio): S/N = 3

Validation of method I

Specificity

First, the specificity of the method was evaluated by injecting the solution containing all solvents from the synthesis route and their potential contaminants at 100% of the limit. In this method benzene at 2 µg/mL level, pyridine at 200 µg/mL level and DMF at 880 µg/mL level (100% of limit) were not found. Then, the specificity of the method was eval-

uated by injecting the specificity solution containing solvents from the synthesis route and their potential contaminants in the following concentrations: methanol (3000 µg/mL), ethanol (5000 µg/mL), acetone (5000 µg/mL), 2-propanol (5000 µg/mL), ACN (410 µg/mL), DCM (600 µg/mL), MTBE (5000 µg/mL), hexane (290 µg/mL), ethyl acetate (5000 µg/mL), THF (720 µg/mL), 2-iodopropane (360 µg/mL), toluene (890 µg/mL) and benzene (200 µg/mL), pyridine (1000 µg/mL), DMF (4400 µg/mL). All peaks in the chromatogram of the specificity solution were completely separated ($R_s = 1.5$), R_s : methanol/ethanol – 13.23, ethanol/acetone – 8.46, acetone/2-propanol – 2.60, 2-propanol/ACN – 3.55, ACN/DCM – 3.68, DCM/MTBE – 5.59, MTBE/hexane – 5.58, hexane/ethyl acetate – 16.87, ethyl acetate/THF – 4.98, THF/benzene – 13.72, benzene/2-iodopropane – 4.46, 2-iodopropane/pyridine – 38.43, pyridine/toluene – 2.22, toluene/DMF – 27.19, DMF/DMA – 20.38 (Figure 1). Spiking the sample solution with the analytes did not cause the peaks to split and the retention times remained the same as for the corresponding peaks from the sample solution.

Linearity

The linearity of the method was evaluated by analyzing six solutions ranging in the concentration of the analytes from about 10% to 120% of the specified limit. All concentrations were prepared in triplicate and the average was reported. The method is linear within a wide range for the solvents included in the validation. The acceptance criteria were confirmed. The results are presented in Table 1.

Range

The results of the solution analyses with the solvent concentrations ranging from 10% to 120% of the specification limit confirmed that the method is precise, linear and accurate. The range: methanol 300 – 3600 µg/mL, acetone 500 – 6000 µg/mL, 2-propanol 500 – 6000 µg/mL, ACN 41 – 492 µg/mL, DCM 60 – 720 µg/mL, ethyl acetate 500 – 6000 µg/mL, THF 72 – 864 µg/mL, 2-iodopropane 36 – 432 µg/mL.

Accuracy

The accuracy of the method was established by assaying 12 sample solutions (triplicate independent preparations of four solutions – the sample solutions spiked with the analytes at about 10%, 50%, 100%, 120% of the specification limit). The results of the mean value of the recovery and RSD are presented in Table 1. The acceptability criteria were fulfilled.

Precision

The precision of the method was established as repeatability, intermediate precision and system suitability. The repeatability was performed by measuring triplicate independent preparations for four solutions – the sample solution spiked with the analytes at about 10%, 50%, 100%, 120% of the specification limit and 6 independent solutions – the sample solution spiked with the analytes at about 100% of the specification limit, then the relative response (the relation of peak area to mass) was calculated. The intermediate precision was repeated on a different day by a different analyst by measuring 6 independent solutions – the sample solution spiked with the analytes at about 100% of the specification

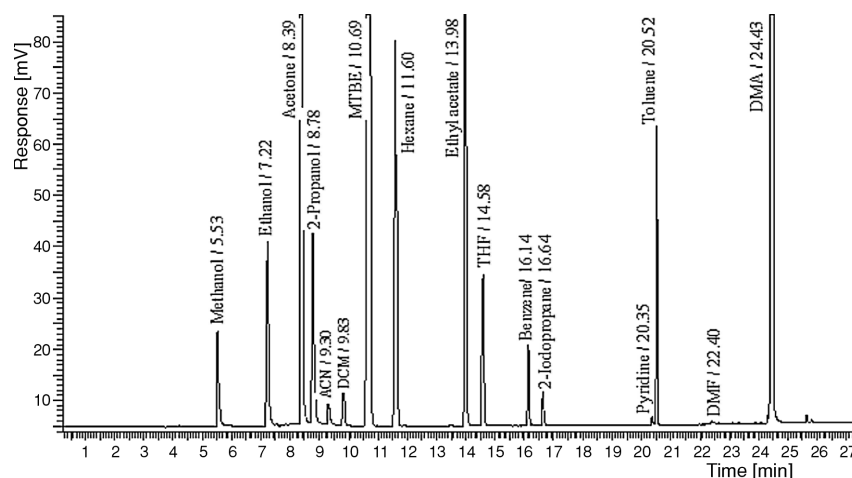


Figure 1. The chromatogram of the specificity solution, methods I and II.

Table 1. Validation results for method I (1 - methanol, 2 - acetone, 3 - 2-propanol, 4 - ACN, 5 - DCM, 6 - ethyl acetate, 7 - THF, 8 - 2-iodopropane).

Test		1	2	3	4	5	6	7	8
Linearity	R ²	0.9990	0.9996	0.9988	0.9993	0.9989	0.9987	0.9986	0.9993
	y-intercept (b)	494.7	-7072.7	-1944.7	-645.9	186.6	787.8	-318.5	-253.2
	t _b	0.37	-1.18	-0.59	2.54	0.39	0.09	-0.16	-0.81
	Slope (a)	36.0	157.1	49.8	63.4	61.8	124.9	201.3	86.1
	t _a	62.74	100.73	58.77	77.85	59.42	53.45	53.45	76.46
	t _r	63.26	99.98	57.77	75.45	55.37	55.37	53.44	75.60
Accuracy	W	0.966	0.858	0.935	0.936	0.937	0.882	0.947	0.902
	Recovery [%]	96.39	103.37	93.20	98.17	98.81	95.72	93.47	102.85
Repeatability	RSD [%]	2.56	6.69	5.41	5.02	1.90	3.58	2.61	2.40
	RSD [%] (Solutions 10% ÷ 120%)	2.57	6.69	5.41	5.02	1.90	3.58	2.61	2.40
Intermediate precision	RSD [%] (Solutions 100%)	1.98	4.44	2.00	2.32	1.41	1.66	2.47	2.63
	RSD [%] (Solutions 100%)	3.37	2.72	2.92	3.00	1.27	1.99	1.95	2.23
Test F-Snedecor	F	2.57	1.84	1.90	1.53	1.31	1.36	1.69	1.13
	RSD [%]	0.67	1.33	0.94	0.93	0.66	0.79	0.64	0.60
System precision	RSD [%] (Solutions 100%)	0.042	0.048	0.018	0.015	0.008	0.017	0.014	0.010
	RSD [%] (Solutions 10%)	4.19	1.07	4.65	8.62	4.78	4.09	4.38	1.52
System precision	RSD [%] (Solutions 10%)	0.040	0.029	0.047	0.056	0.036	0.016	0.021	0.022

R² - correlation coefficient; t_b, t_r, t_r - para meters of Student's t-test; W - parameter of Shapiro-Wilk test; RSD - relative standard deviation; t_g - retention time

limit, then the relative response (the relation of peak area to mass) was calculated. The comparison of the repeatability and intermediate precision results was performed using the F-Snedecor test. The system suitability was established by measuring the response of six replicate injections of the standard solution with the analytes at 100% of the specification limit) and six replicate injections of the standard solution with the analytes at 10% of the specification limit). The results are expressed as the RSD and summarised in Table 1. All criteria were fulfilled.

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The limit of quantitation (LOQ) and limit of detection (LOD) were evaluated by using the standard solutions containing known low concentrations of the solvents. The concentration which generated the peak about 10 times as high as the noise's height was established as the LOQ (methanol – 60 µg/mL, acetone – 20 µg/mL, 2-propanol – 70 µg/mL, ACN – 32 µg/mL, DCM – 24 µg/mL, ethyl acetate – 19 µg/mL, THF – 9 µg/mL, 2-iodopropane – 20 µg/mL with respect to the sample preparation). The concentration which generated the peak about 3 times as high as the noise's height was established as the LOD (methanol – 20 µg/mL, acetone – 5 µg/mL, 2-propanol – 26 µg/mL, ACN – 8 µg/mL, DCM – 7 µg/mL, ethyl acetate – 9 µg/mL, THF – 3.2 µg/mL, 2-iodopropane – 4 µg/mL with respect to the sample preparation).

Robustness

The robustness of the method was evaluated by injecting the specificity solution to ensure the separation of all solvents from the synthesis route using

different chromatographic conditions. The following parameters were tested: column temperature $\pm 5^{\circ}\text{C}$, rate $\pm 1^{\circ}\text{C}/\text{min}$, carrier gas pressure $\pm 10\%$ and constant temperature time $\pm 1\text{ min}$. The smallest resolution ($R_s = 1.90$) was obtained between acetone and 2-propanol at a higher temperature of the column ($50 - 65 - 145^{\circ}\text{C}$). Changes in the analytical conditions did not influence the resolution significantly and the method was robust.

Validation of method II

Specificity

The specificity of the method was evaluated in the same way as for Method I. The results are presented in section 3.2.1.

Limit of Detection (LOD)

The prepared solutions containing known low concentrations of the solvents were injected into the chromatograph. The concentration which generated the peak about 3 times as high as the noise's height was established as the LOD (ethanol – 30 µg/mL, MTBE – 1 µg/mL, hexane – 0.32 µg/mL, toluene – 1.4 µg/mL with respect to the sample preparation).

System suitability

The system suitability was established by measuring the response of six replicate injections of the standard solution with the analytes at 100% of the specification limit and six replicate injections of the standard solution with the analytes at 10% of the specification limit. The results are expressed as the RSD and summarised in Table 2. All criteria were fulfilled.

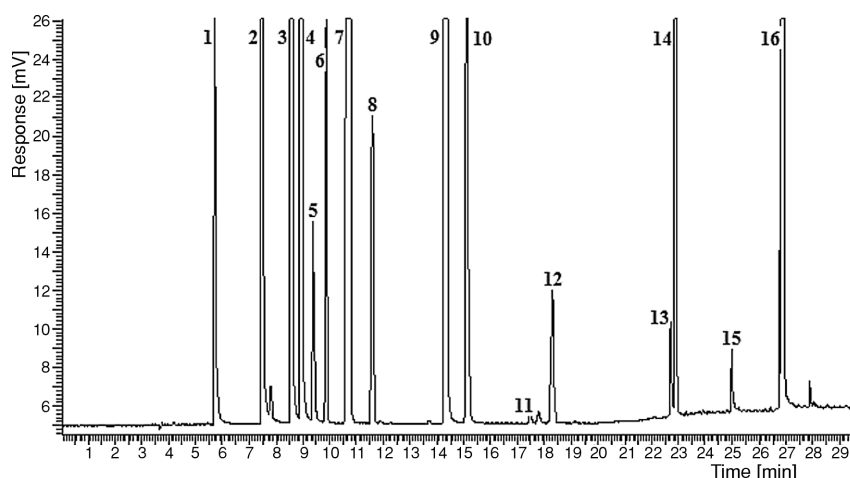


Figure 2. The chromatogram of the specificity solution, method III (1 – methanol, 2 – ethanol, 3 – acetone, 4 – 2-propanol, 5 – ACN, 6 – DCM, 7 – MTBE, 8 – hexane, 9 – ethyl acetate, 10 – THF, 11 – benzene, 12 – 2-iodopropane, 13 – pyridine, 14 – toluene, 15 – DMF, 16 – DMA).

Validation of method III

Specificity

The specificity of the method was evaluated by injecting the specificity solution containing solvents from the synthesis route and their potential contaminants at 100% of the limit: methanol, ethanol, acetone, 2-propanol, ACN, MTBE, dichloromethane, n-hexane, ethyl acetate, tetrahydrofuran, toluene, benzene, 2-iodopropane and pyridine (2000 µg/mL), DMF (3%). All peaks in the chromatogram of the specificity solution were completely separated ($R_s = 1.5$) from benzene, R_s : THF/benzene – 14.89, benzene/2-iodopropane – 4.75 (Fig. 2).

Limit of Detection (LOD)

The prepared samples containing known low concentrations of benzene were injected into the chromatograph. The concentration which generated the peak about 3 times as high as the noise's height was established as the LOD. In this method, the LOD was 0.3 µg/mL with respect to the sample preparation.

System suitability

The system suitability was established by measuring the response of six replicate injections of the standard solution with benzene at 100% of the

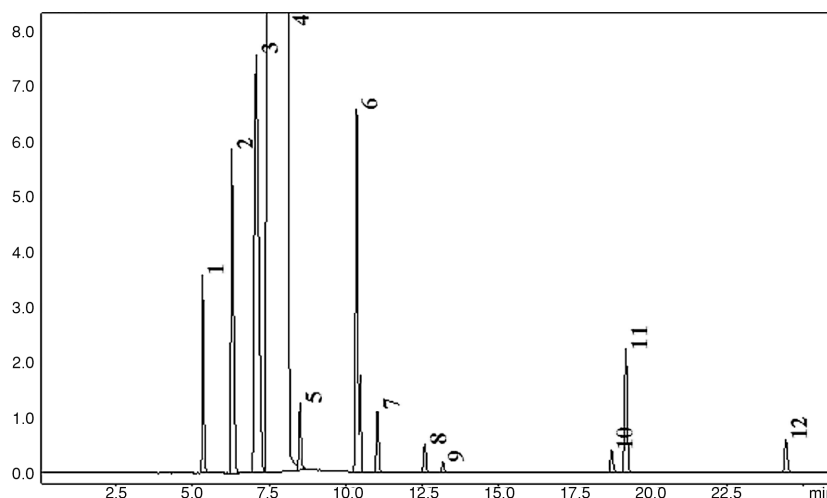


Figure 3. The chromatogram of the specificity solution, method IV (1 – methanol, 2 – ethanol, 3 – acetone + 2-propanol, 4 – ACN + DCM + MTBE, 5 – hexane, 6 – ethyl acetate, 7 – THF, 8 – benzene, 9 – 2-iodopropane, 10 – pyridine, 11 – toluene, 12 – DMF).

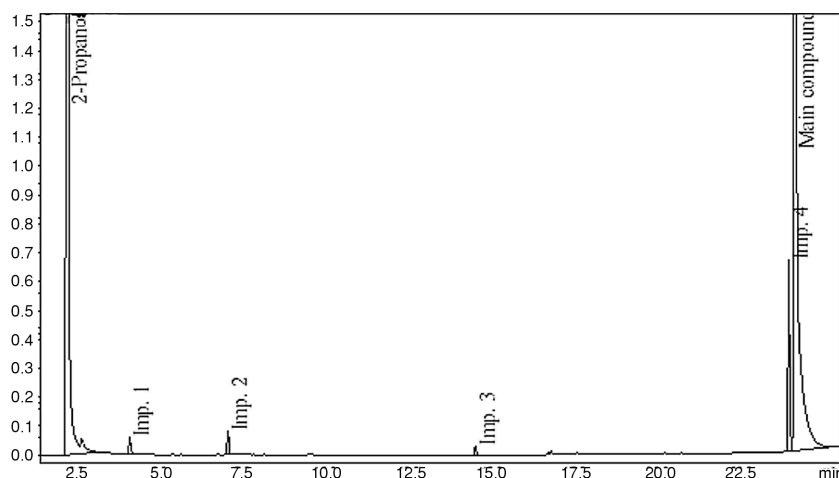


Figure 4. The chromatogram of the sample solution, method V.

Table 2. System precision results for methods II, III and IV.

		Solution with the analytes at 10% or 30% of the specification limit		Solution with the analytes at 100% of the specification limit	
		Peak area RSD [%]	t _R RSD [%]	Peak area RSD [%]	t _R RSD [%]
Method II	Ethanol	3.18	0.023	1.70	0.057
	MTBE	1.32	0.027	1.33	0.026
	Hexane	2.31	0.017	1.94	0.009
	Toluene	1.56	0.009	1.44	0.010
Method III	Benzene	2.42	0.046	4.68	0.057
Method IV	Pyridine	3.45	0.120	4.48	0.109
	DMF	5.01	0.045	3.52	0.032

RSD - relative standard deviation; t_R - retention time

specification limit (2 µg/mL) and six replicate injections of the standard solution with benzene at 30% of the specification limit (0.6 µg/mL). The results are expressed as the RSD and summarised in Table 2. All criteria were fulfilled.

Validation of method IV

Specificity

The specificity of the method was evaluated by injecting the specificity solution containing solvents from the synthesis route and their potential contaminants at 100% of the limit: methanol, ethanol, acetone, 2-propanol, MTBE, dichloromethane, n-hexane, ethyl acetate, tetrahydrofuran, pyridine, toluene, 2-iodopropane, DMF and benzene (200 µg/mL). All peaks in the chromatogram of the specificity solution were completely separated ($R_s = 1.5$) from the analytes (pyridine and DMF), R_s : 2-iodopropane/pyridine – 36.76, pyridine/toluene – 3.01, toluene/DMF – 32.34 (Fig. 3).

Limit of Detection (LOD)

The prepared solutions containing known low concentrations of solvents were injected into the chromatograph. The concentration which generated the peak about 3 times as high as the noise's height was established as the LOD (pyridine – 4 µg/mL, DMF – 10 µg/mL with respect to the sample preparation).

System suitability

The system suitability was established by measuring the response of six replicate injections of the standard solution with the analytes at 100% of the specification limit) and six replicate injections of the standard solution with the analytes at 10% of the specification limit). The results are expressed as the RSD and summarised in Table 2. All criteria were fulfilled.

Validation of method V

During the purity determination of the starting material, impurities presented in the tested substance (including four main impurities: impurity 1 t_R = 4.1 min; impurity 2 t_R = 7.1 min; impurity 3 t_R = 14.5 min; impurity 4 t_R = 23.9 min) were separated from the main compound (t_R = 24.1 min).

Specificity

The specificity of this method was examined by injecting 2-propanol and the sample solution (100% of the nominal concentration). The method was specific for the main compound and its main impurities (R_s : 2-propanol/impurity 1 – 22.7, impurity 1/impurity 2 – 30.9, impurity 2/impurity 3 – 83.9, impurity 3/ impurity 4 – 115.7, impurity 4/main compound – 2.4). The chromatogram of the sample solution is shown in Fig. 4.

Linearity

The linearity of the method was evaluated by measuring the response of five solutions ranging in the concentrations from about 5% to 120% of the nominal concentration. The method is linear within a wide range for the main compound and impurities. The acceptance criteria were confirmed. The results are presented in Table 3.

Precision

The precision was established by measuring the response of six replicate injections of the sample solution. The RSDs were calculated for the retention time, peak area, assay (%) and are presented in Table 3. All criteria were fulfilled.

Range

The range was established by analyzing the solutions in the range of 20-120% of the nominal

concentration. The analysis of the solutions has confirmed that the purity test results are repeatable and simultaneously the method is linear for the main compound and impurities 1- 4. The range in which the area normalization method is correct: 20% ÷ 120 % of the nominal concentration in the sample solution. The RSDs are presented in Table 3.

Detection limit

The detection limit (LOD) was calculated as the concentration which generated the peak of the main compound about 3 times as high as the noise's height (0.01% of the nominal concentration in the sample solution).

CONCLUSIONS

Impurities are often not completely removed by practical manufacturing techniques and consequently their low levels are present in most pharmaceuticals. The determination of OVI in pharmaceutical products is probably the most important application of GC in quality control in the pharmaceutical industry (10).

Five simple, sensitive, specific and precise GC methods for the determination of residual volatile compounds from the synthesis route of travoprost and the purity determination of the starting material TV-38A were developed and validated. A complete validation of the quantitative test procedure to control the presence of the specified solvents used in the last step of the travoprost synthesis (methanol, acetone, 2-propanol, acetonitrile, dichloromethane, ethyl acetate, tetrahydrofuran, and 2-iodopropane) was performed. This GC-HS method turned out to be specific, accurate, linear and precise. The sol-

vents were detected and quantified at a µg/mL level. The validation of the limit test procedure for the control of unspecified solvents (a GC-HS method for ethanol, methyl *t*-butyl ether, hexane, toluene and a GC method with direct injection for pyridine and DMF) used prior to the last step of the travoprost synthesis and not detected in the tested batches of the substance demonstrated adequate specificity, precision and allowed the µg/mL detection. The validation of the limit test procedure for the control of benzene (potential contaminant of acetone and toluene, not detected in the tested batches of the substance) also demonstrated adequate specificity, precision and allowed the µg/mL detection. The validation results of the gas chromatographic method with the area normalization method for the calculation, used for the purity control of the starting material TV-38A, are specific, linear, sensitive and precise. The validation results clearly demonstrate that the analytical procedures are suitable for their intended purpose. Five presented gas chromatographic methods may well be used for the routine QC analysis of the travoprost API and its starting material TV-38A.

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Table 3. Validation results for method V.

Test			Imp. 1	Imp. 2	Imp. 3	Imp. 4	Main compound
Linearity	R ²		0.9999	0.9992	0.9999	0.9996	1
	y-intercept (b)		-118.9	-160.7	129.9	315.5	14513
	t _b		-1.52	-0.44	2.63	0.18	1.24
	Slope (a)		233.9	300.6	102.2	2067.4	61473
	t _a		217.9	59.6	150.7	83.4	380.8
	t _r		223.5	59.7	151.9	83.5	387.5
Precision	RSD [%]	t _R	0.068	0.044	0.025	0.017	0.016
	RSD [%]	Area	0.18	0.36	0.42	2.05	0.16
	RSD [%]	Assay%	0.25	0.40	0.43	2.06	0.07
Range	RSD [%]	Assay%	2.38	3.12	2.33	1.17	0.08

R² - correlation coefficient; t_a, t_b, t_r - parameters of Student's t-test; RSD - relative standard deviation; t_R - retention time

Conflict of interests

The authors declare no conflict of interest.

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