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SHORT COMMUNICATIONS

DEVELOPMENT AND VALIDATION OF ANALYTICAL PROCEDURE – CONTROL OF RESIDUAL 2-IODOPROPANE IN LATANOPROST

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Potential genotoxic impurities used in the synthesis of pharmaceutical substances should be identified based on the existing genotoxicity data or the presence of structural alerts and then determined by suitable analytical techniques (1). 2-Iodopropane, a halogenated aliphatic compound, has been used during the synthesis of latanoprost (2) and could be recognized as a potential genotoxic impurity. In this study, elements of validation of GC method of determination of 2-iodopropane in latanoprost are presented. Validation of the method included: selectivity, system precision, method precision, accuracy (recovery), limits of detection and quantitation (in substance), robustness and linearity.

Specification limit has been established for 2-iodopropane at the level of 1000 ppm.

EXPERIMENTAL

Equipment and conditions

Gas chromatograph (Shimadzu GC-2010) with flame-ionization detector, DB-624 column (60 m long, 0.32 mm ID, phase composition: 6% cyanopropylphenyl – 94% dimethylpolysiloxane).

Column temperature: 100°C, ramp 2°C/min to 130°C, ramp 40°C/min to 240°C, 10 min at final temperature, injecton port temperature: 240°C, detector temperature: 260°C, carrier gas: nitrogen, pressure: 100 kPa, split ratio: 8 : 1, inject: 1 µL.

Standard solution

Accurately weighed ca. 100 mg of 2-iodopropane was introduced into a 25 mL volumetric flask which contains ca. 10 mL of dimethyl sulfoxide (DMSO). Half mL of this solution was transferred into a 10 mL volumetric flask, the volume was completed with DMSO and mixed. Standard solution was made from 0.5 mL of this standard stock solution which was introduced into a 10 mL volumetric flask, completed to volume with DMSO and mixed.

Sample preparation

Test solution: accurately weighed ca. 10 mg of the examined substance was placed into 1 mL volumetric flask and filled up to volume with DMSO, then mixed thoroughly.

RESULTS

The method was validated by the determination of the following parameters: selectivity, system precision, method precision, accuracy (recovery), limits of detection and quantitation (in substance), robustness and linearity.

Selectivity

The selectivity of the method was proved using the solution consisting of solvents from the synthesis route. Chromatogram of this solution is shown in Figure 1. The resolution values are presented in Table 1. The acceptance criterion (resolution = 1.5) was satisfied.

System precision

System precision was established by measuring the response of six replicate injections of the standard solution. The results are presented in Table 2. The acceptance criterion (RSD = 10%) was satisfied.

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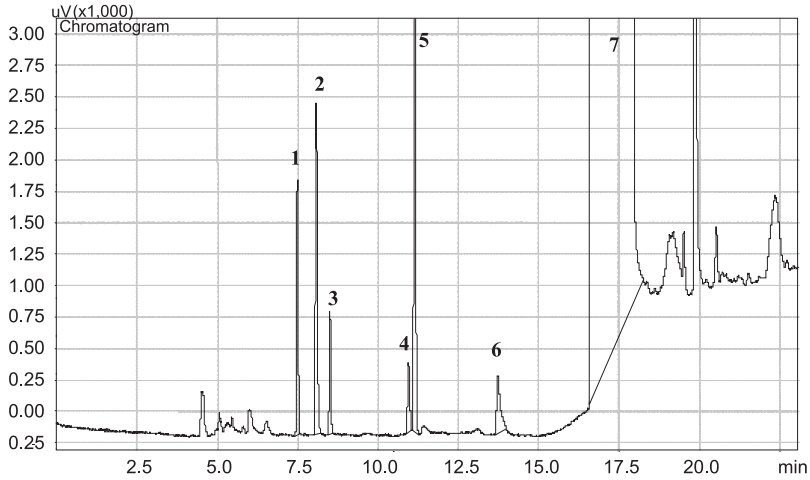

Figure 1. Selectivity chromatogram. 1 – Tetrahydrofuran; 2 – Triethylamine; 3 – 2-Iodopropane; 4 – Pyridine; 5 – Toluene; 6 – Dimethylformamide; 7 – DMSO

Table 1. Selectivity

Compound name	Retention time (min)	Resolution between solvents
Tetrahydrofuran / Triethylamine	7.500 / 8.085	6.420
Triethylamine / 2-Iodopropane	8.085 / 8.515	4.430
2-Iodopropane / Pyridine	8.515 / 10.961	22.577
Pyridine / Toluene	10.961 / 11.154	1.643
Toluene / Dimethylformamide	11.154 / 13.746	17.334
Dimethylformamide / DMSO (solvent of tested substance and standards)	13.746 / 17.868	5.622

Table 2. Precision

Sample no.	System precision Response	Method precision Relative response	Intermediate precision Relative response
1	3574	31.02	34.88
2	3725	30.66	33.98
3	3541	29.47	31.89
4	3043	30.67	33.49
5	3479	30.29	39.23
6	3437	30.01	37.29
Mean	3467	30.35	35.13
SD	229.92	0.555	2.69
RSD%	6.63%	1.83%	7.64%

Table 3. Accuracy (recovery)

Compound	Recovery (%)
Three independent samples spiked with 2-iodopropane at 50% of specification limit	102.6 98.7 96.2
Three independent samples spiked with 2-iodopropane at 100% of specification limit	88.8 87.7 86.9
Three independent samples spiked with 2-iodopropane at 120% of specification limit	86.0 90.7 85.5
Mean	91.5
SD	6.19
RSD%	6.55%
Confidence interval	86.7% – 96.3%

Table 4. Robustness

Parameters of the method	Resolution between analytes	
	Triethylamine / 2-Iodopropane	2-Iodopropane / Pyridine
Standard method	4.097	20.731
Carrier gas pressure 90 kPa	4.625	22.128
Carrier gas pressure 110 kPa	4.229	20.987
Column temperature 95 – 125°C	4.328	22.600
Column temperature 105 – 135°C	4.502	19.939
Rate 1°C/min	4.386	22.821
Rate 3°C/min	4.564	22.789
Another column (US7167763H)	4.390	21.393

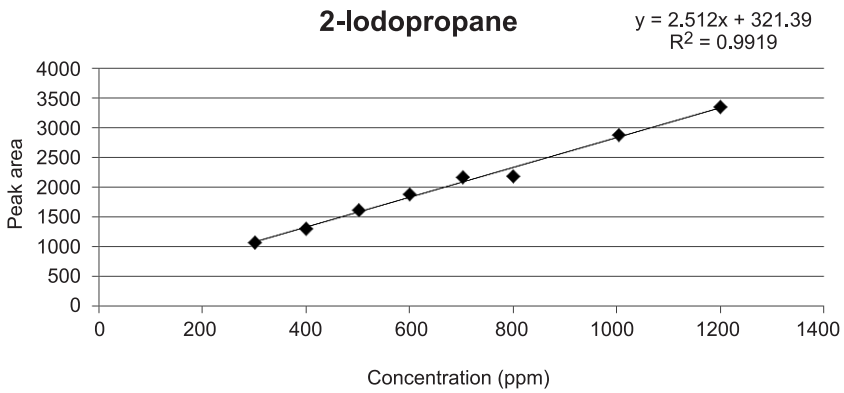

Figure 2. Linearity of the method

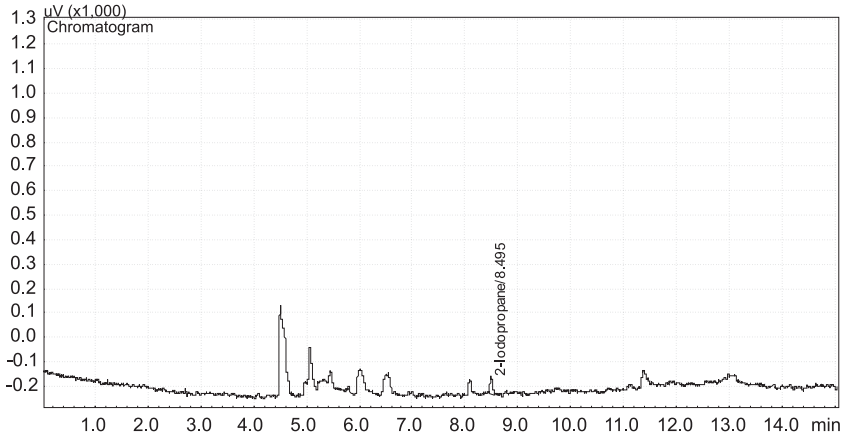

Figure 3. Concentration of 2-iodopropane 80.6 ppm (substance)

Method and intermediate precision

The method precision was established by measuring six independent test solutions spiked with 2-iodopropane at 100% of specification, then the relative responses (relation of peak area to mass) were calculated. The method precision determination was repeated on another day by another analyst (intermediate precision). The results are presented in Table 2. The acceptance criterion (RSD = 15%) was satisfied.

Accuracy (recovery)

The accuracy of the method was examined with the use of the test solution and 9 independent samples spiked with 2-iodopropane at 50%, 100% and 120% of specification limit (triplicate preparations for each solutions) against standard solution. The results are presented in Table 3. The acceptance criteria (Recovery 80% ÷ 120%, RSD = 15%) were satisfied.

Linearity

The linearity of 2-iodopropane concentrations was evaluated by analyzing solutions ranging in concentrations from ca. LOQ to 120% of the specification limit with respect to sample preparation. Three replicate injections for each concentration were made. The results are presented in Figure 2. The acceptance criterion ($R^2 = 0.98$) was satisfied.

Quantitation and detection limits

Solutions of different lowering concentrations of 2-iodopropane were injected into chromatograph. The concentration which generated peak about 10 times as high as the noise's height was stated as LOQ (quantitation limit). The concentration which generated peak about 3 times as high as the noise's height was stated as LOD (detection limit). The limit of quantitation was 300 ppm and the limit of detection was 80 ppm. The chromatogram of 2-iodopropane solution close to LOD concentration is shown in Figure 3.

Robustness

The robustness of the method was evaluated to ensure the separation of the solvents with the use of different chromatographic conditions. The resolution between determined solvents was calculated and is presented in Table 4. The following parameters were changed: carrier gas pressure ± 10 %, column temperature ± 5^{o}C, rate ± 1^{o}C/min, other column (same type, other batch). The acceptance criterion (resolution = 1.5) was satisfied.

CONCLUSION

Validation results demonstrate that the analytical procedure – Control of residual 2-iodopropane in latanoprost – is suitable for its intended purpose. All the parameters fall within the limits desired for the method.

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