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DETERMINATION OF ORGANIC VOLATILE IMPURITIES IN NEPAFENAC BY GC METHOD

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Abstract: The methods for controlling volatile impurities, including reagent and starting material, in Nepafenac active pharmaceutical ingredient, are reported. The proposed methods were developed using gas chromatography (GC) and gas chromatography with headspace injection (GC-HS) and validated according to the requirements of the ICH (International Conference of Harmonization) validation guidelines Q2R1 and the guideline for residual solvents Q3C.

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

Nepafenac, 2-amino-3-benzoylbenzeneacetamide (NF3), is non-steroidal anti-inflammatory drug (NSAID) formulated as an ophthalmic suspension. Nepafenac is a pro-drug of amfenac, a highly effective nonselective cyclooxygenase COX-1 and COX-2 inhibitor. It is used to prevent and treat ocular pain and inflammation that can occur after cataract surgery by reducing the production of prostaglandins in the eye (1, 2). Recently, our new method for nepafenac synthesis (Fig. 1.) and HPLC validated procedure for its purity and assay determination has been published (3, 4). To continue our studies on nepafenac active pharmaceutical ingredient, the GC and GC-HS methods for determination of organic volatile impurities and residual solvents has been elaborated and verified in validated analytical procedure. It is commonly known that residual solvents in active pharmaceutical ingredient are used or produced in manufacturing process and may stem from starting materials, reagents, intermediates, solvents etc. or can be formed in degradation process. It is assumed that the solvents and other volatile impurities may not be fully removable during API manufacturing. Therefore, their acceptable amounts are recommended due to patient safety and elimination of potential toxic risk (5, 6). Moreover, the presence of the particular solvent in production process effects the yield and quality of API and drug formulation. Thus, the selection of the solvents may be considered as one of the critical parameter of the process. According to *ICH Topic Q3C (R4) Impurities: Guideline for Residual Solvents* all sol-

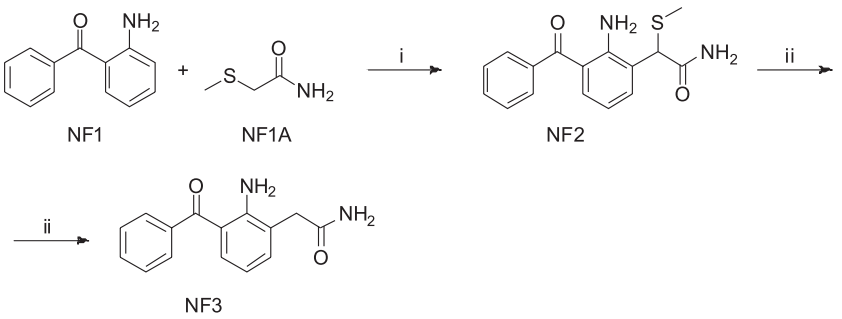

Figure 1. Nepafenac synthesis: i) N-chlorophthalimide, 1,4-dichloromethane (DCM), triethylamine (TEA), 1 M KOH_{aq} ii) Raneys type nickel, acetone, 2-propanol

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vents are classified into four classes based on their toxicity and potential environmental hazard (Class 1 – should be avoided; Class 2 – should be limited in pharmaceutical products because of their inherent toxicity; Class 3 – should be used only where it would be impractical to remove them and Class 4 – there is no adequate toxicological data and manufacturers should supply justification for the residual levels of these solvents in pharmaceutical products).

In the official monographs: USP (United States Pharmacopeial Convention) and EP (European Pharmacopoeia) there are no documents related to nepafenac. There are also no references to GC studies for the determination of: residual solvents, TEA and NF1A in active substance or purity control of the starting material NF1A, except the GC method disclosed for residual solvents in the patent document (7).

According to the method presented in this patent, the analyses were carried out using the gas chromatograph with FID, interfaced an auto-sampler and chromatographic separations were performed on the TRB-5 column. The main disadvantages of revealed procedure was the long analysis time.

In the present study, the residual solvents in nepafenac active substance were determined, using gas chromatographic techniques with headspace injection (GC-HS). For controlling the quality of the starting material NF1A (Fig. 1.) and the level thereof in final nepafenac API as well as triethylamine residual reagent, the gas chromatography methods with direct injection appeared to be more effective. The all revealed validation results meet the requirements of the ICH (International Conference of Harmonization) validation guidelines Q2R1 (5) and the guideline for residual solvents Q3C (6). Our analytical studies include the following validated methods: the full validation of a GC-HS analytical method for the determination of residual solvents (Class 3: acetone, 2-propanol); the limited validations of GC-HS analytical methods for the determination of solvents (Class 2: dichloromethane, methanol, toluene and Class 1: benzene); the limited validations of GC methods with direct injection analytical for the determination of triethylamine (TEA) and NF1A; the validation of the GC method with direct injection to control the quality of the starting material – NF1A (normalization method procedure).

MATERIALS AND METHODS

Chemicals and reagents

The active substance – nepafenac was synthesized at Pharmaceutical Research Institute (Warszawa, Poland). The solvents and starting material were purchased from commercial suppliers (acetone, 2-propanol, methanol, dichloromethane and toluene from POCH Avantor Performance Materials Poland S.A. (Poland), benzene and triethylamine from Fluka (Germany), N,N-dimethylacetamide from Sigma-Aldrich (Germany), 2-(methylthio)acetamide from Watson International Ltd. (China)).

Preparation of solutions and sample

All solutions were prepared directly before the analysis. In the methods I, II, IV and V the blanks were made up of N,N-dimethylacetamide (DMA); in the method III blank solution was formed from N,N-dimethylacetamide (DMA) and 1 mL of H_2O; and in the method VI the blank contained only methanol.

Method I (Determination of acetone, 2-propanol)

The test solution was prepared by dissolving the appropriate amounts of nepafenac in DMA to obtain the concentration of 4%. The standard solution I was prepared by dissolving the appropriate amounts of acetone and 2-propanol in DMA and then dilution to reach 5000 µg/mL of acetone and 2-propanol with respect to the sample preparation. The standard solution II was prepared by diluting the standard solution I to reach 500 µg/mL of acetone and 2-propanol.

Method II (Determination of methanol, dichloromethane and toluene)

The test solution was prepared by dissolving the appropriate amounts of nepafenac in DMA to obtain the concentration of 4%. The standard solution was prepared by dissolving the appropriate amounts of methanol, dichloromethane and toluene in DMA and dilution to reach 300 µg/mL of methanol, 60 µg/mL of dichloromethane and 89 µg/mL of toluene with respect to the sample preparation.

Method III (Determination of benzene)

The test solution was prepared by dissolving the appropriate amounts of nepafenac in DMA to obtain the concentration of 10%. The standard solution was prepared by dissolving the appropriate amounts of benzene in DMA and dilution to reach 0.6 µg/mL with respect to the sample preparation.

Method IV (Determination of triethylamine)

The test solution was prepared by dissolving the appropriate amount of nepafenac in DMA to obtain the concentration of 8%. The standard solu-

tion was prepared by dissolving the appropriate amount of triethylamine in DMA and dilution to reach 500 µg/mL with respect to the sample preparation. The reference solution was prepared by adding the standard solution to the sample.

Method V (Determination of 2-(methylthio)acetamide)

The test solution was prepared by dissolving the appropriate amount of nepafenac in DMA to obtain the concentration of 8%. The standard solution was prepared by dissolving the appropriate amount of 2-(methylthio)acetamide (NF-1A) in DMA followed by dilution up to 500 µg/mL with respect to the sample preparation. The reference solution was prepared by addition the standard solution to the sample.

Method VI (Purity control of the starting material NF-1A)

The test solution was prepared by dissolving the appropriate amount of 2-NF-1A in methanol to obtain the concentration of 10%.

Chromatographic conditions

Methods: I, II and III were performed using the Perkin Elmer CLARUS 500 gas chromatograph with a flame ionization detector interfaced with a Perkin Elmer headspace TURBOMATRIX 40 autosampler. Chromatographic separations were performed on a DB-624 column (phase composition: 6% cyanopropylphenyl – 94% dimethylpolysiloxane), film thickeness 1.8 µm, 60 m long and 0.32 mm ID. Method IV was carried out on the Shimadzu GC-2010 gas chromatograph with a flame ionization detector interfaced with a Shimadzu AOC-20i autosampler. Chromatographic separations were performed on a DB-624 column (phase composition: 6% cyanopropylphenyl – 94% dimethylpolysiloxane), film thickeness 1.8 µm, 60 m long, 0.32 mm ID. The other two methods (V and VI) were conducted on the same apparatus as Method IV, with the difference being that DB-5 column was used for chromatographic separations (phase composition: (5% phenyl methylpolysiloxane – 95% dimethylpolysiloxane), film thickeness 1.0 µm, 30 m long, 0.32 mm ID)

Method I and Method II (Determination of acetone, 2-propanol and methanol, dichloromethane, toluene)

These two methods used the same oven temperature programs: the initial temperature of 45°C was maintained for 9 min after the injection; then, it was ramped up at the rate of 10°C/min to 150°C and ramped up again at the rate of 40°C/min to 240°C, finally maintained for 3 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, injection: 0.05 min.

Method III (Determination of benzene)

The oven temperature program was as follows: the initial temperature 35°C was ramped up at the rate of 2°C/min to 70°C and it was maintained for 2 min then, it was ramped up 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, injecton: 0.07 min.

Method IV (Determination of triethylamine)

The oven temperature program was as follows: the initial temperature of 35°C; it was then ramped up at the rate of 2°C/min to 70°C and maintained for 3 min then, it was ramped up again at the rate of 40°C/min to 240°C and maintained for 10 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, injection 1 µL.

Method V (Determination of NF-1A)

The oven temperature program was as follows: the initial temperature of 120°C; it was ramped up at the rate of 10°C/min to 190 °C, then, ramped up again at the rate of 30°C/min to 280°C and maintained for 10 min. The injection port temperature was 260°C and the detector temperature was 290°C. Nitrogen was used as the carrier gas at 50 kPa, split 10 : 1, injection 1 µL.

Method VI (Purity control of the starting material NF-1A)

The oven temperature program was as follows: the initial temperature of 60°C; it was then ramped up at the rate of 5°C/min to 260°C. The injection port temperature was 260°C and the detector temperature was 290°C. Nitrogen was used as the carrier gas at 50 kPa, split 20:1, injection 0.5 µL.

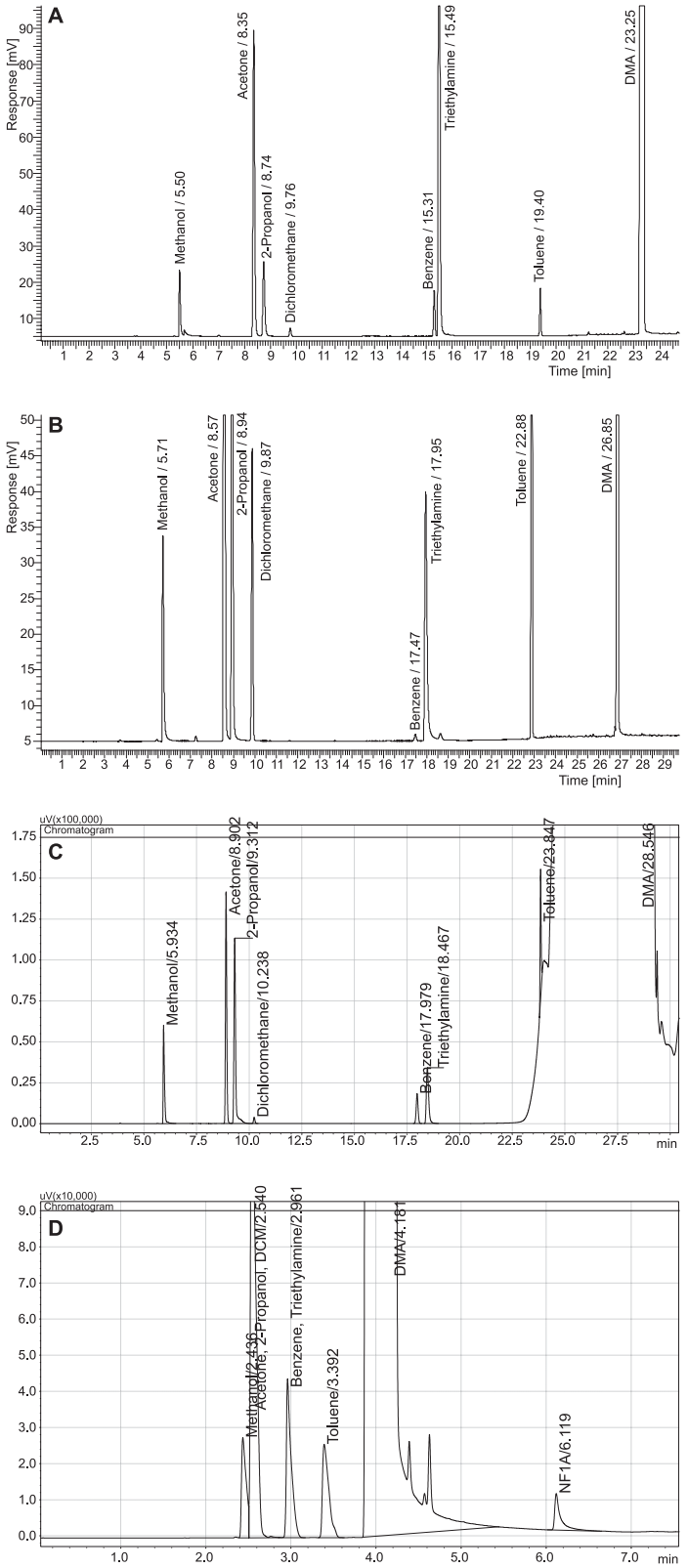

Figure 2. The chromatograms of the specificity solution: **A**) methods I and II; **B**) method III; **C**) method IV; **D**) method V

RESULTS AND DISCUSSION

Method I (Determination of acetone, 2-propanol – full validation procedure)

In this study, the HS-GC analytical method was developed and validated for the quantitative determination of acetone and 2-propanol i.e., the solvents used in the last step of the nepafenac synthesis. The method selectivity, limits of detection and quantitation, linearity, range, precision (system repeatability), recovery and robustness (changes in the GC conditions) were determined in consequence.

Specificity

The following solvents were used during the synthesis of nepafenc: acetone, 2-propanol, methanol, dichloromethane, toluene, triethylamine and a potential contaminant of acetone – benzene. The specificity of the method was evaluated by injecting the specificity solution containing the solvents from the synthesis route. The method was spe-

Table 1. Results of the method I (linearity, precision, SST, accuracy).

Parameters		Acetone	2-Propanol
Linearity of the method			
Statistical parameters of regression	R	0.9998	1.0000
	R^2	0.9995	0.9999
	y-intercept (b)	1504.6	–1218.3
	S_b	3685.08	423.05
	$t_{b, exp.}$	0.41	–2.88
	Slope (a)	77.819	21.852
	S_a	0.986	0.113
	$t_{a, exp.}$	78.95	193.02
	$t_{kr} = 3.18_{(\alpha = 0.05, n=5)}$	$t_r = 77.22$	$t_r = 173.2$
	y = ax + b	y = 77.819x + 1504.6	y = 21.852x – 1218.3
Precision			
Results of the precision	SD %	2.81%	2.60%
Results of 6 independent reference solutions	RSD %	4.98%	2.57%
Results of 6 independent reference solutions intermediate precision	RSD %	2.54%	2.28%
F-Snedecor test	F	3.31	1.10
System precision (SST)			
The solution containing 5000 µg/mL of the analytes	RSD% Peak area	1.66%	1.52%
	RSD% Retention time (min)	0.018%	0.017%
The solution containing 500 µg/mL of the analytes	RSD% Peak area	4.07%	4.14%
	RSD% Retention time (min)	0.027%	0.023%
Accuracy			
Recovery	[%]	105.33	108.63
RSD	[%]	2.81	2.72
CI	–	1.88	1.88

R – correlation coefficient, t_a , t_b , t_r , t_{kr} – parameters of Student's t-test, S_a, S_b – standard deviation of a and b, CI – confidence interval, RSD – relative standard deviation, F – parameters of Snedecor's F-test, SST – system suitability test.

cific for residual solvents (resolution R_s = 1.5; methanol/acetone – 27.28, acetone/2-propanol – 2.90, 2-propanol/dichloromethane – 8.01, dichloromethane/benzene – 51.08, benzene/triethylamine – 1.75, triethylamine/toluene – 39.66, toluene/DMA – 28.21). Spiking the sample with the analyte did not cause the peak splitting and the retention times remained the same as for the corresponding peak from the test solution (Fig. 2.).

Linearity and range

The linearity of the method was evaluated by analyzing five solutions ranging in concentrations from about 500 to 6000 µg/mL with respect to sample preparation. 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; acceptance criteria (R^2 = 0.990, $y = ax + b$, $t_a = t_{kr,} | t_b | < t_{kr} , | t_r | > t_{kr}$ (α = 0.05, n – 2)) were confirmed. The plot of the concentration *versus* the response, the correlation coefficient, y-intercept and slope of the regression line were calculated and are presented in Table 1.

Precision

The precision of the method was established as repeatability, system and intermediate precision. Repeatability was performed by measuring triplicate independent preparations of four solutions – sample spiked with the analytes at 500, 2500, 5000 and 6000 µg/mL with respect to the sample preparation; 3 test solutions and 6 independent solutions – sample spiked with the analytes at about 5000 µg/mL with respect to the sample preparation, 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 – sample spiked with the analytes at about 5000 µg/mL with respect to the sample preparation, then the relative response (the relation of peak area to mass) was calculated. The comparison of the repeatability results and intermediate precision were performed using the F-Snedecor test. The results are expressed as a relative standard deviation (RSD%) and summarized in Table 1 and all criteria were fulfilled (RSD = 15%, $F = F_{kr}$ (α = 0.05, $f_1 = n_1 - 1$, $f_2 = n_2 - 1$) F_{kr} = 5.05 (n = 6)).

SST (system suitability test)

The system suitability test involves the examination of the system precision and resolution. The system precision was established by measuring the response of six replicate injections of the standard solution I and six replicate injections of the standard solution II. The results are presented as a relative standard deviation (RSD%) for the peak area and retention time (Table 1) and these were below 10% (peak area) and 1% (retention time).

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 the known low concentrations of solvents. The concentration which generated the peak about 10 times as high as the noise's height was stated as LOQ (acetone – 28 µg/mL, 2-propanol – 90 µg/mL). The concentration which generated the peak about 3 times as high as the noise's height was stated as LOD (acetone – 9 µg/mL, 2-propanol – 35 µg/mL).

Robustness

The robustness of the method was evaluated by injecting the specificity solution to ensure the separation of all the solvents from synthesis route with the use of different chromatographic conditions. The following parameters were tested: column temperature ± 5°C, rate ± 1°C/min, carrier gas pressure ± 10% and constant temperature time ± 1 min. The smallest resolution (Rs) was obtained between benzene and triethylamine Rs = 1.53 at carrier gas pressure 90 kPa. The changes in analytical conditions did not influence the resolution significantly and the method was robust.

Accuracy

The accuracy of the method was established by assaying 12 sample solutions (triplicate independent preparations of four solutions – sample spiked with the analytes at 500, 2500, 5000 and 6000 µg/mL of the specification limit). The results of the recovery, relative standard deviation (RSD%) and confidence interval (CI) are presented in Table 1. The acceptable criteria were set up as the RSD value below 15% and the recovery: 80–120%.

Method II (Determination of methanol, dichloromethane and toluene – limited validation procedure)

The control of residual methanol, dichloromethane and toluene in nepafenac by GC-HS method was elaborated as a limit test procedure, because these solvents were used prior to the last step in the synthesis and were not detected in the tested batches of the substance. The validation of this method included the examination of specificity, detection limit and additionally system precision.

Specificity

The specificity of the method was evaluated by injecting the specificity solution consisting of the solvents from the synthesis route (Fig. 2). The method was specific for residual solvents (R_s = 1.5, methanol/acetone – 27.28, acetone/2-propanol – 2.90, 2-propanol/dichloromethane – 8.01, dichloromethane/benzene – 51.08, benzene/triethylamine – 1.75, triethylamine/toluene – 39.66, toluene/DMA – 28.21).

SST (system suitability test)

The system precision was established by measuring the response of six replicate injections of the SST solution with the solvents at 300 µg/mL of methanol, 60 µg/mL of dichloromethane and 89 µg/mL of toluene with respect to the sample preparation. Results were presented as a relative standard deviation (RSD%) for the peak area (methanol – 1.46%, dichloromethane – 6.05%, toluene – 5.35%) and retention time (methanol – 0.025%, dichloromethane – 0.023%, toluene – 0.010%) and these were below 10% (peak area) and 1% (retention time).

Limit of detection (LOD)

The prepared solutions containing known low concentrations of solvents were injected into a chromatograph. The concentration which generated the peak about 3 times as high as the noise's height was stated as LOD (methanol – 28 µg/mL, dichloromethane – 25 µg/mL, toluene – 8 µg/mL).

Method III (Determination of benzene)

The validation of this method (limit test procedure) included the examination of the specificity, system precision and the detection limit. Benzene was not used during the synthesis but it is commonly considered as a potential contaminant of acetone and toluene. In the tested batches of nepafenac benzene was not detected.

Specificity

The specificity of the method was evaluated by injecting the specificity solution consisting of the solvents from the synthesis route (Fig. 2). The method was specific for residual solvents (R_s = 1.5, methanol/acetone – 23.81, acetone/2-propanol – 3.01, 2-propanol/dichloromethane – 7.49, dichloromethane/benzene – 53.06, benzene/triethylamine – 2.65, triethylamine/toluene – 36.44, toluene/DMA – 47.59).

SST (system suitability test)

The system precision was established by measuring the response of six replicate injections of the solution with benzene at the level of 0.6 µg/mL, with respect to the sample preparation. The results were presented as a relative standard deviation (RSD%) for the peak area and retention time and were: RSD% (peak area) – 4.54%; , RSD% (retention time) – 0.033%, respectively to acceptance criteria (RSD = 10% – peak area, RSD = 1% retention time).

Limit of detection (LOD)

The prepared samples containing known low concentrations of benzene were injected into a chromatograph. The concentration which generated the peak about 3 times as high as the noise's height was stated as LOD. In this method LOD was found as 0.2 µg/mL, with respect to the sample preparation.

Method IV (Determination of triethylamine)

The validation of a GC analytical method (limit test procedure) with direct injection for the determination of TEA involved the examination of the specificity, system precision as well as the detection limit, because triethylamine was used during the synthesis but not detected in the tested batches of nepafenac.

Specificity

The specificity of this method was examined by the use of the specificity solution. The specificity solution consists of the solvents from the synthesis route: acetone, 2-propanol, methanol, dichloromethane, toluene, triethylamine and benzene. The parameter measured included the resolution (Rs) and it was higher than 1.5 (methanol/acetone – 27.45, acetone/2-propanol – 3.34, 2-propanol/dichloromethane – 7.22, dichloromethane/benzene – 54.26, benzene/triethylamine – 2.71, triethylamine/toluene – 38.27, toluene/DMA – 12.91). The chromatogram of this solution is shown in Figure 2.

Limit of detection (LOD)

The determination of the signal-to-noise was performed by comparing the measured signals from the prepared samples containing known low concentrations of TEA with those of the blank samples and establishing the minimum concentration at which TEA can be reliably detected. The concentration which generated the peak about 3 times as high as the noise's height was stated as LOD and it amounted to 4 µg/mL (substance).

SST (system suitability test)

The system suitability test was analyzed by measuring the response of six replicate injections of

the reference solution (TEA at the level of 500 µg/mL, with respect to the sample preparation). The results were presented as a relative standard deviation (RSD%) for the peak area and retention time and were: RSD% (peak area) – 0.96%; RSD% (retention time) – 0.017%, respectively to acceptance criteria (RSD = 10% – peak area, RSD = 1% retention time).

Method V (Determination of NF-1A)

The method was validated for the examination of the specificity, system precision as well as the detection limit (limit test procedure), because NF-1A was used during the synthesis but not detected in the tested batches of nepafenac.

Specificity

The specificity of this method was examined by the use of the specificity solution. The specificity solution consists of the solvents from the synthesis route: acetone, 2-propanol, methanol, dichloromethane, toluene, triethylamine and a potential contaminant of acetone – benzene. The parameter measured included the resolution (Rs) and it was higher than 1.5 (methanol + acetone + 2-propanol + dichloromethane + benzene + triethylamine + toluene + DMA /NF1A – 8.38). The chromatogram of this solution is shown in Figure 2.

Limit of detection (LOD)

The determination of the signal-to-noise is performed by comparing the measured signals from the prepared samples containing known low concentrations of NF-1A with those of the blank samples and establishing the minimum concentration at which NF-1A can be reliably detected. The concentration which generated the peak about 3 times as high as the noise's height was stated as LOD and it was 180 µg/mL (substance).

SST (system suitability test)

The system precision was established by measuring the response of six replicate injections of the reference solution (NF-1A at level 500 µg/mL, with respect to the sample preparation). The results were presented as a relative standard deviation (RSD%) for the peak area and retention time and were: RSD% (peak area) – 3.95%; RSD% (retention time) – 0.031%, respectively to acceptance criteria (RSD = 10% – peak area, RSD = 1% retention time).

Method VI (Purity control of the starting material NF-1A)

The gas chromatography method with direct injection was applied to control the quality of this material. The validation of this method (normalization method procedure) included tests of the specificity, detection limit, linearity and range at the area normalization.

Specificity

The specificity of this method was examined by the use of the test solution. The parameter measured included resolution (Rs) and it was higher than 1.5 (methanol/impurity 1 – 7.6, impurity 1/impurity of methanol – 6.1, impurity of methanol/impurity 2

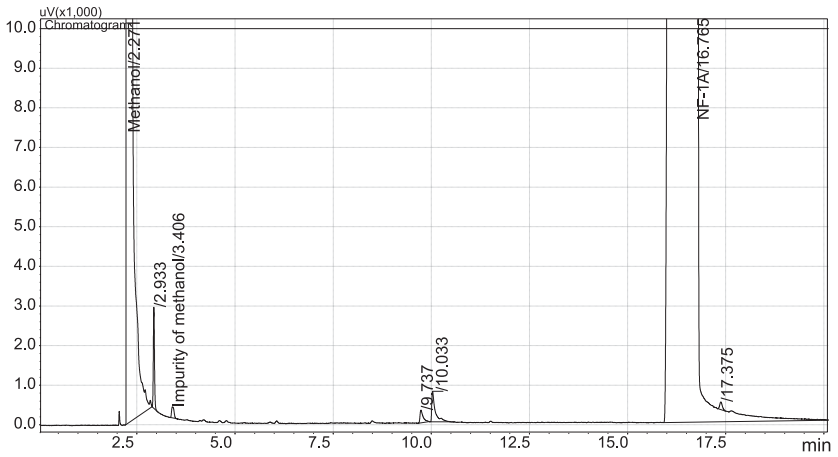

Figure 3. The chromatogram of test solution – method VI

Table 2. Results of the method VI (linearity and range of the area normalization method).

Parameters					**2-(methylthio)acetamide**
Linearity of the method					
Statistical parameters of regression		R R^2 y-intercept (b) S_b $t_{b, exp.}$ Slope (a) S_a $t_{a, exp.}$ $t_{kr} = 3.18_{(\alpha = 0.05, n=5)}$ y = ax + b			0.9997 0.9994 –20968 117209 –0.18 108419 1588 68.26 $t_r = 70.70$ y = 108419x – 20968
Range of the area normalization method					
Solution	Concentration (% of test solution)	Impurity 1 [%]	Impurity 2 [%]	Impurity 3 [%]	NF-1A [%]
1	120%	0.046 0.046	0.017 0.017	0.041 0.044	99.895 99.894
2	100%	0.047 0.045	0.016 0.016	0.043 0.042	99.894 99.897
3	50%	0.045 0.044	0.014 0.015	0.037 0.037	99.904 99.905
4	10%	0.017 0.012	0 0	0.025 0.025	99.958 99.964
Mean		0.038	0.012	0.037	99.914
SD		0.014	0.007	0.008	0.029
RSD		36.84%	58.33%	21.62%	0.03%
1	120%	0.046 0.046	0.017 0.017	0.041 0.044	99.895 99.894
2	100%	0.047 0.045	0.016 0.016	0.043 0.042	99.894 99.897
3	50%	0.045 0.044	0.014 0.015	0.037 0.037	99.904 99.905
Mean		0.046	0.016	0.041	99.898
SD		0.001	0.001	0.003	0.005
RSD		2.17%	6.25%	7.32%	0.005%

R – correlation coefficient, t_a , t_b , t_r , t_{kr} – parameters of Student's t-test, S_a, S_b – standard deviation of a and b.

– 52.8, impurity 2/impurity 3 – 2.3, impurity 3/NF-1A – 14.4, impurity 4 – on the tail of the main peak). The chromatogram of this solution is shown in Figure 3.

Limit of detection (LOD)

The solutions of different lowering concentrations of the examined substance were injected into a chromatograph. The concentration which generated the peak about 3 times as high as the noise's height was stated as LOD. In this method LOD was 0.05%.

Linearity

The linearity of the method was evaluated by analyzing the solutions ranging in concentrations from about 5 to 120% of the test solution. The method is linear within this range and acceptance criteria [$R^2 = 0.990$, y = ax + b , $t_a = t_{kr,} | t_b | < t_{kr ,} | t_r | > t_{kr} (\alpha = 0.05, n – 2)$] were confirmed. Two replicate injections were made for each concentration and the average result was reported. The correlation coefficient, y-intercept and slope of the regression line were calculated (Table 2).

Range of the area normalization method

The range of the area normalization method was evaluated by analyzing the solutions ranging in concentrations from about 120 to 10% of the test solution. Two replicate injections were made for each concentration. The acceptance criteria of this method: RSD = 15% for impurities; RSD = 0.05% for NF-1A were suitable for its intended purpose. The results of the assay (%) of the impurities and NF-1A, the mean, standard deviation (SD) and relative standard deviation (RSD%) are presented in Table 2.

DISCUSSION AND CONCLUSION

In the present report, a simple, rapid, sensitive, reliable, specific, accurate and precise GC methods for the determination of residual solvents (acetone, 2-propanol, methanol, toluene, dichloromethane, benzene), reagent (triethylamine) and starting material (2-(methylthio)acetamide) in nepafenac API batches and purity of starting material (NF-1A) were developed and validated. The complete validation of the GC-HS method to control the presence of solvent from the final synthetic step, acetone and 2-propanol, in nepafenac API was performed. The method turned out to be specific, accurate, linear, precise and the solvents were detected and quantified at a µg/mL level. Similarly, GC-HS limit test procedure for the solvents used in manufacturing process but not observed in nepafenac batches, i.e., methanol, dichloromethane, toluene and benzene as the potential contaminant of acetone, demonstrated adequate specificity, precision and allowed for the µg/mL detection. In course of triethylamine (reagent) and starting material NF-1A evaluation in nepafenac batches it was shown that GC analytical method with direct injection is suitable for its intended purpose i.e., limit test procedure. The satisfying results were achieved for the purity control of the starting material NF-1A. In conclusion, all the parameters for the demonstrated analytical methods fall within the expected limits, therefore, GC-HS and GC methods can be used for the routine QC analysis of nepafenac API.

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