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VALIDATION OF GC METHOD FOR QUANTITATIVE DETERMINATION OF RESIDUAL 2-(2-CHLOROETHOXY)ETHANOL (CEE) AND N-METHYL-2-PYRROLIDINONE (NMP) IN PHARMACEUTICAL ACTIVE SUBSTANCE

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Abstract: The gas chromatography method with direct injection for quantitative determination of residual non-volatile solvents such as 2-(2-chloroethoxy)ethanol (CEE) and N-methyl-2-pyrrolidinone (NMP) in quetiapine – the pharmaceutical active substance has been validated. Validation was performed according to the requirement of ICH validation guidelines Q2A and Q2B. Specificity, precision, accuracy, linearity, limits of detection and quantitation and robustness were determined and excellent results were obtained.

Keywords: 1-methyl-2-pyrrolidone (NMP), 2-(2-chloroethoxy) ethanol (CEE), GC method, validation, quetiapine

Quetiapine, 2-{2-(4-dibenzo[*b,f*][1,4]thiazepine-11-yl-1-piperazinyl)ethoxy}ethanol fumarate salt, is an antipsychotic drug acting as 5-HT_{2A} antagonist and dopamine D_2 antagonist, which has fewer side effects and better activity than the classical antipsychotic agents. It has been used for treatment of schizophrenia, depressive symptoms in patients with primary psychotic disorders (schizophrenia and schizo-affective disorder) and psychotic mood disorders (bi-polar disorder and major depressive disorder). New medical uses of quetiapine are claimed in patent applications as treatment of human suffering from bulimia nervosa, treatment of weight, treatment of attention deficit, hyperactivity disorder, treatment of substance abuse and dyskinesias associated with dopaminergic or levodopa therapy.

It is known that some solvents and starting materials are often not totally removed by practical manufacturing techniques, and consequently low levels are present in most pharmaceutical substances. The European Pharmacopoeia (Ph. Eur.) (1) describing a general procedure for Identification and Control of Residual Solvents, classified solvents into three classes on the basis of the toxicity level and the degree to which they can be considered an environmental hazard (2), in a drug substance. The pharmacopoeial procedure describes a limit test for the quantitation of solvents from 1st and 2nd class, which is suitable for a routine control, however, during the development of a drug substance (changes in process, scale-up, etc.) the accurate quantitation is necessary. The implementation of this general method is a subject of major concern in the pharmaceutical industry, especially during the quantitative determination of non-volatile solvents such as 2-(2-chloroetoxy)ethanol (CEE) and N-methyl-2-pyrrolidinone (NMP). NMP is classified into Class 2 with a permissible daily exposure (PDE) of 5.3 mg/day (530 ppm) (3). CEE is an unclassified substance but as a known impurity could be specified with acceptance criterion 0.15% (4). The development of gas chromatographic method with direct-injection for quantitative determination of residual CEE and NMP in pharmaceutical active substance has been described in our earlier work (5).

In this study, the validation of this method, according to ICH (International Conference on Harmonisation of Technical Requirements for Registrations of Pharmaceuticals for Human Use) requirements Q2A and Q2B (6, 7), is presented.

EXPERIMENTAL

Chemicals

Chemicals of an analytical grade were used for the validation. The active substance – quetiapine,

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was synthesised in PRI (Pharmaceutical Research Institute, Warszawa, Poland). N,N-dimethylformamide (DMF) was purchased from Merck (Darmstadt, Germany), 1-methyl-2-pyrrolidone (NMP) was provided by Sigma-Aldrich (Steinheim, Germany) and 2-(2-chloroetoxy)ethanol (CEE) was provided by Maruzen Chemical (Japan).

Chromatographic conditions

Chromatographic separations was performed on DB-624 column (phase composition: 6% cyanopropylphenyl – 94% dimethylpolysiloxane) film thickeness 1.8 µm, 60 m long, 0.32 mm ID. The experiments were performed on a Shimadzu GC-2010 gas chromatograph (GC) equipped with a Shimadzu AOC -20i autosampler and a flame ionization detector (FID).

GC parameters: inlet heater 240°C, detector 260°C, oven initial temperature 150°C, then raised at a rate of 5°C/min to 240°C. Nitrogen was used as a carrier gas at 120 kPa (constant flow, approximately 1.56 mL/min) and a split flow of 5 mL/min. Air flow rate of 400 mL/min and hydrogen flow rate of 47 mL/min were used for FID. For injection 1 µL was used.

Solutions

All solutions were prepared directly before analysis.

Standard stock solutions were prepared by dissolving appropriate amounts of CEE and NMP in DMF to reach: Standard stock solution A containing 1000 µg/mL of CEE, Standard stock solution B containing 1000 µg/mL of NMP. Standard solution was prepared by the appropiate dilution of stock solutions to reach: 50 µg/mL of CEE and 26.5 µg/mL of NMP which corresponds to 1000 µg/mL of CEE and 530 µg/mL of NMP in the tested substance.

Test solution was prepared by dissolving appropriate amounts of quetiapine in DMF to obtain concentration of 5 %. To obtain clear solution, the ultrasonic bath was applied during 10 min. Blank solution consisted only from DMF used for standard and test solution preparation.

Validation solutions were prepared as follows: Solution I: was the test solution containing 40 µg/mL of CEE and 21 µg/mL of NMP, which corresponds to 800 µg/mL of CEE and 420 µg/mL of NMP in the tested substance. Solution II: was the test solution containing 50 µg/mL of CEE and 26.5 µg/mL of NMP, which corresponds to 1000 µg/mL of CEE and 530 µg/mL of NMP in the tested substance. Solution III: was the test solution containing 60 µg/mL of CEE and 32 µg/mL of NMP, which corresponds to 1200 µg/mL of CEE and 640 µg/mL of NMP in the tested substance.

Chromatographic procedure

Separately, 1 µL of standard solution and test solution were injected into gas chromatograph, each of them 2 or 3 times. Peak areas of recorded chromatograms were compared for analytes from the test and standard solutions. In descibed conditions the retention time was about 9 min and about 11 min for CEE and NMP, respectively.

The mean area of the CEE peak in the chromatograms obtained from the test solution should not be greater than the mean area of the CEE peak obtained from the standard solution (1000 µg/mL in the substance), whereas that of the NMP peak should not be greater than the mean area of the NMP peak obtained from the standard solution (530 µg/mL in the substance).

The concentration of these analytes is calculated from the equation:

$$\text{Residual solvent in µg/mL} = \frac{c_{std} \times A_{test}}{A_{std} \times c_{test}} \times 10^6$$

where: c_{std} = concentration of residual solvents in standard solution, % w/v; c_{test} = concentration of tested substance in test solution, % w/v; A_{std} = mean peak area of analytes in the chromatogram of the standard solution; A_{test} = mean peak area of analytes in the chromatogram of the test solution.

Evaluation

In order to establish the validation parameters, the peak area (x), mean peak area($\overline{x}$), relative standard deviation (RSD), and confidence interval as: $\overline{x} \pm \Delta x$ were evaluated.

The recovery was calculated using the following formula (1):

$$\text{Recovery} = \frac{W_{std} \times A_{sol}}{A_{std} \times W_{sol}} \times 100\% \qquad (1)$$

where: W_{std} = weight of analytes in mg in 1 mL of the standard solution; W_{sol} = weight of analytes in mg in 1 mL of the solution I, II or III; A_{std} = peak area of analytes in the chromatogram of the standard solution; A_{sol} = peak area of analytes in the chromatogram of the solution I, II or III.

For intermediate precision the Snedecor's F-tests were performed using the following formula (2):

$$F = \frac{SD_1^2}{SD_2^2} \qquad SD_1 > SD_2 \qquad (2)$$

where: SD_1 = standard deviation from the results obtained by the first analyst; SD_2 = standard devi-

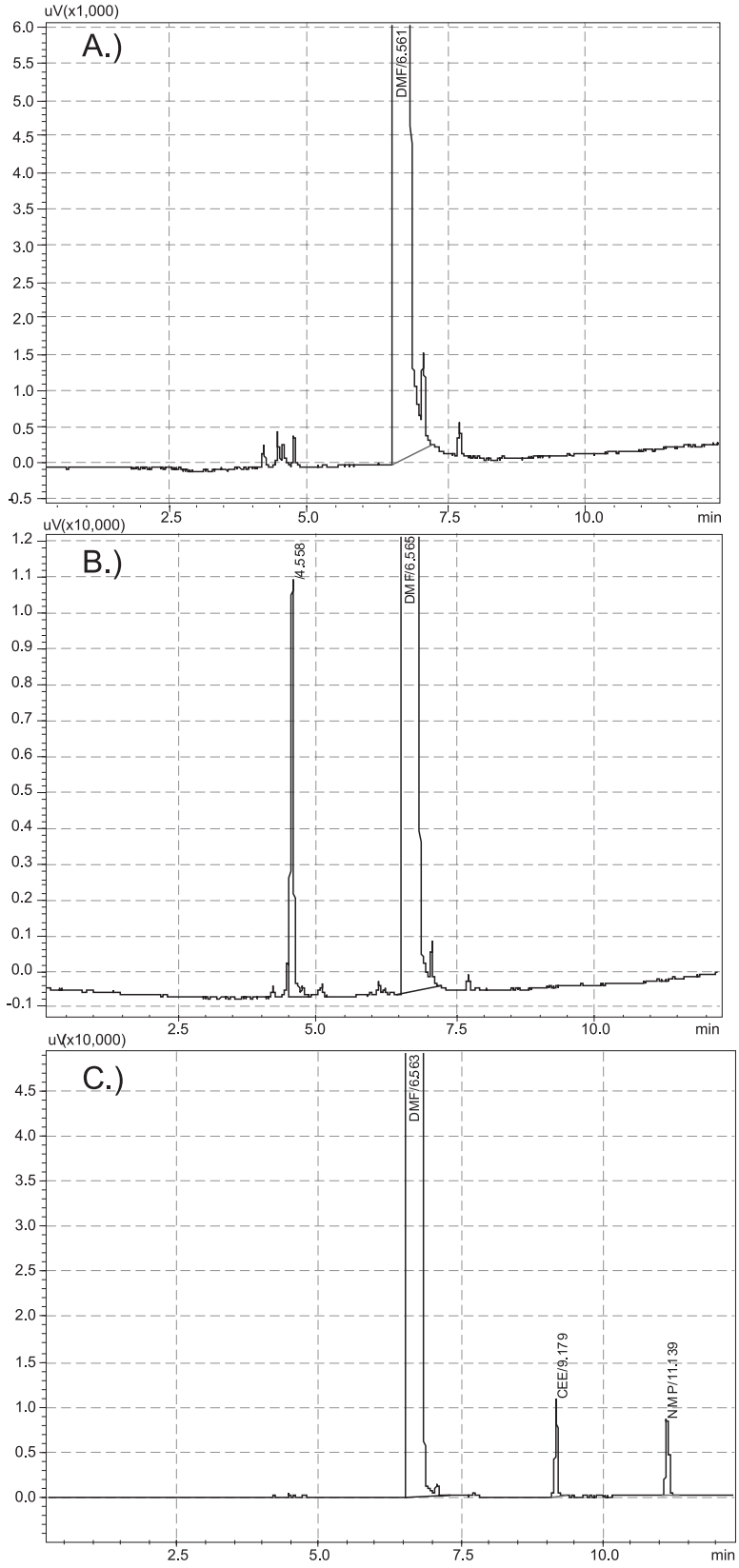

Figure 1. The comparison of chromatograms obtained from A.) blank solution, B.) test solution and C.) standard solution

ation from the results obtained by the second analyst.

RESULTS AND DISCUSSION

The validation procedure was performed based on the ICH requirements. During the validation, the specificity, precision, linearity, accuracy, limits of detection (LODs) and quantitation (LOQs) and robustness were investigated.

Specificity

During the synthesis of quetiapine the following solvents were used: ethanol, acetone, toluene, dichloromethane, NMP, CEE. The selectivity of the method concerning the analytes in relation to other solvents was shown in previous work (5). The specificity of the method was showed using blank solution (DMF), test solution, test solution spiked with CEE (1000 µg/mL in the tested substance), test solution spiked with NMP (530 µg/mL in the tested substance) and standard solution (CEE and NMP in DMF). Peak area in the chromatogram of the solution spiked with analyte was greater than corresponding peak area in the chromatogram of the test solution. Spiking the sample with analyte did not cause the peak splitting – retentions times stayed the same as for corresponding peak from test solution. In the chromatogram of blank solution there were no observed peaks with retention times of CEE and NMP.

In Figure 1, the comparison of chromatograms obtained from blank solution, test solution and standard solution is presented. The peak presented in Figure 1B, whose retention time is about 4.6 min., derives from the sum of remaining solvents in the tested sample.

Precision

The precision of the method was established as repeatability, system and intermediate precision. Repeatability was performed for 6 independent Solutions II prepared as described above. System precision was established by measuring the response of six replicate injections of the standard solution. Intermediate precision was established by perform-

Table 1. System, method and intermediate precision for CEE and NMP.

No.	Peak area (x), µV		Statistical parameters for CEE	Statistical parameters for NMP
	CEE	NMP		
System precision				
1 2 3 4 5 6	35519 35206 35446 35101 35400 34891	32195 31972 32483 31959 32062 31869	$\bar{x}$ = 35261 SD = 239.01 RSD = 0.68 % $\bar{x} \pm \Delta x$ = 35261 ± 251	$\bar{x}$ = 32090 SD = 221.89 RSD = 0.69 % $\bar{x} \pm \Delta x$ = 32090 ± 233
Method precision (first analyst)				
1 2 3 4 5 6	35236 34692 33888 33832 33446 32689	34186 33859 33133 33610 33083 32939	$\bar{x}$ = 33964 SD = 901.68 RSD = 2.66 % $\bar{x} \pm \Delta x$ = 33964 ± 946	$\bar{x}$ = 33468 SD = 495.75 RSD = 1.48 % $\bar{x} \pm \Delta x$ = 33468 ± 520
Method precision (second analyst)				
	CEE	NMP		
1 2 3 4 5 6	39150 39099 37164 38884 37825 36837	36180 36373 35187 37755 37244 36687	$\bar{x}$ = 38160 SD = 1024 RSD = 2.68 % $\bar{x} \pm \Delta x$ = 38160 ± 1075	$\bar{x}$ = 36571 SD = 892 RSD = 2.44 % $\bar{x} \pm \Delta x$ = 336571 ± 936
Intermediate precision (results from 1st and 2nd analyst)			SD_1 = 1024 SD_2 = 901.68 $F_{5,5,exp.}$ = 1.29	SD_1 = 892 SD_2 = 495.75 $F_{5,5,exp.}$ = 3.24

Table 2. Accuracy for CEE and NMP.

No.	Recovery		Statistical parameters for CEE	Statistical parameters for NMP
	CEE	NMP		
I	105.06 % 104.22 % 103.85 %	99.41 % 98.02 % 99.49 %	$\bar{x}$ = 101.12 %	$\bar{x}$ = 101.13 %
II	104.73 % 104.59 % 99.42 %	102.40 % 102.94 % 99.59 %	SD = 4.27 RSD = 4.22 % $\bar{x} \pm \Delta x$ = 101.12 ± 3.28	SD = 1.98 RSDV = 1.96 % $\bar{x} \pm \Delta x$ = 101.13 ± 1.53
III	98.41 % 95.48 % 94.32 %	103.48 % 102.60% 102.21 %		

Table 3. Statistical parameters for linearity of CEE and NMP.

CEE				
No.	Concentration (µg/mL)	Peak area, µV	Statistical parameters of regression	
1 2 3 4 5 6 7	0.68 4.89 9.77 24.43 39.08 48.85 58.62	410 3157 6468 16871 27697 34783 41706	R^2 S_r y-intercept S_b $t_{b,exp.}$ slope S_a $t_{a,exp}$	0.9999 208990 -359.21 124.51 2.89 717.08 3.67 195.51
NMP				
1 2 3 4 5 6 7	0.37 2.66 5.32 13.30 21.28 26.60 31.92	383 3048 6326 16294 26723 33430 40110	R^2 S_r y-intercept S_b $t_{b,exp.}$ slope S_a $t_{a,exp.}$	0.9999 127376 -298.36 97.19 3.07 1265.67 5.26 240.62

Table 4. Effect of changes of GC conditions on system suitability.

Variations			Results			
Pressure (kPa)	Initial T (^oC)	Split flow (mL/min)	Resolution DMF/CEE	Resolution CEE/NMP	RSD for CEE (%)	RSD for NMP (%)
120	150	5	15.35	21.58	0.68	0.69
120	160	5	12.15	17.83	0.89	0.93
110	150	5	14.53	21.70	1.67	1.81
120	150	7.5	16.98	11.23	1.56	1.44

ance the repeatability test on another day by another analyst. Intermediate precision was determined by comparison of the results obtained by both analysts, using Snedecor's F-test.

The acceptance criteria were set up as RSD value below 3%, 6% and 10%, respectively. An additional criterium based on Snedecor's F-test was set up as: if $F_{experimental} \leq F_{critical}$ (for $\alpha = 0.05$, $f_1 =$

n_1-1, $f_2 = n_2-1$) the difference between the results obtained by both analysts is insignificant. Critical parameter F ($\alpha = 0.05$; $f_1, f_2 = 5$) is 5.05. The results of method precision are presented in Table 1 and pointed out that all criteria were fulfilled and the method is precise.

Accuracy

Accuracy was assessed on samples spiked with known amounts of CEE and NMP (from 80 % to 120 % of specified limit). The accuracy of the method was established by measuring nine sample solutions (triplicate preparations for solution I, II and III) against standard solution, then the recovery results were calculated. The acceptance criteria were set up as RSD value below 10% and recovery value 100% ± 10%. The recovery results, are presented in Table 2. The set up criteria were fulfilled, thus, the method is accurate.

Limits of detection and quantitation

The sensitivity of the method was demonstrated by the low LOD values obtained for CEE and NMP. The limit of detection (LOD) calculated as the concentration, which generated a peak about 3 times as high as the noise's height, and the limit of quantitation (LOQ) calculated as the concentration, which generated peak about 10 times as high as the noise's height, were found as 3 µg/mL and 10 µg/mL for CEE and 2.2 µg/mL and 7 µg/mL for NMP, respectively.

Linearity

The linearity of the method was evaluated by performance of 3 replicates of standard solutions at seven concentration levels in a range between LOQ and 120% of the specification limit. Concentrations of CEE ranged from 0.68 – 58.62 µg/mL, which corresponds to approximately 13.7 – 1172 µg/mL in the tested substance. Concentrations of NMP ranged from 0.37 – 31.92 µg/mL, which corresponds to approximately 7.5 – 638 µg/mL in the tested substance.

The results of statistical evaluation of the linearity experiments (correlation coefficient, y-intercept, slope of regression line, residual sum of squares (S_r)) are summarized in Table 3. The obtained correlation coefficients (r^2) of linear regression for both solvents were above 0.999. These indicates a linear relationship between the concentrations of analytes and the response of detector. Critical parameter t (95%, 5) is 2.571. Our results show that parameters a and b are statistically important and our method is characterized by very good precision and is free from systematic errors.

Robustness

In order to evaluate robustness of the method, the influence of variations of method parameters such as pressure, temperature and split were investigated. System suitability (SST) requirements were checked for variations of ±10 kPa on the carrier gas flow, ±10°C on the initial oven temperature and ±50% on the split ratio. For each set of variation, six replicate injections of the standard solution were performed. The obtained results are presented in Table 4 and indicate that studied variations of GC conditions do not cause any significant changes in system suitability and the method is robust.

Conclusions

In this study, the validation of GC method for control of residual 1-methyl-2-pyrrolidone (NMP) and 2-(2-chloroethoxy)ethanol (CEE) in quetiapine were performed. During the validation procedure, carried out according to ICH guidelines Q2A and Q2B (6, 7), specificity, precision, accuracy, limits of detection and quantitation and robustness were evaluated. All set up criteria were fulfilled. The method is specific, accurate, linear and shows a satisfactory level of precision. The determined solvents can be detected and quantified at µg/mL level. The validation procedure shows that the method is suitable for its intended purpose.

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