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APPLICATION OF NEW DATA PROCESSING METHOD FOR THE PHOTODIODE ARRAY DETECTOR IN THE DRUG SUBSTANCE ANALYSIS

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While analyzing active pharmaceutical ingredients (APIs) it is necessary to determine both the assay of the drug substance itself and the content of impurities in a drug substance. The ideal situation is when both assay of drug substance and content of related substances can be determined simultaneously (1). However, as the impurities must be determined at relatively low levels, it is often impossible to select such analytical conditions, which allow to determine both the API assay and the content of impurities in one chromatographic run. Drug substance concentration in a method for the determination of its purity is often outside the linear range of the applied detector and an additional analytical procedure for the drug substance assay must be developed (2, 3).

An Intelligent Dynamic Range Extension Calculator (i-DReC) is a new data processing method for the photodiode array detector, available in the LabSolutions software by Shimadzu. It allows to extend the linear range of chromatographic method by shifting the chromatographic profile to the wavelength where the target signal is not saturated. The peak area of the acquired chromatogram is corrected by the factor of the absorption ratio between the original target wavelength and the wavelength used by the i-DReC function. The i-DReC is applied automatically when the intensity of the target peak exceeds the user-defined threshold value (4, 5).

Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo-[5,1-*d*]-as-tetrazine-8-carboxamide; TZ) is an oral alkylating drug used for the treatment of primary brain tumors (6, 7). The temozolomide signal is saturated in the method for the determination of temozolomide related substances used for the assessment of the chemical purity of temozolomide samples manufactured by Pharmaceutical Research Institute (PRI), which imposed the necessity of using separate methods for the temozolomide API assay and purity determination (2).

The aim of the study was to apply the i-DReC data analysis function of the LabSolutions software to the determination of temozolomide assay using a method for the chemical purity determination. It was possible in spite of the temozolomide signal being saturated.

EXPERIMENTAL

Reagents and equipment

Acetonitrile (ACN) and methanol (MeOH) of HPLC grade (Super Gradient) were obtained from POCh (Poland). Acetic acid (HAc, > 99.8%) was purchased from Fluka (Germany). Demineralized water (< 0.0055 µS/cm) from Polwater system (Poland) was used throughout the investigation. All analyses were performed using the UHPLC Shimadzu Nexera X2 with LC-30AD pumps, an SIL-30AC autosampler, a CTO-20AC column oven, a DGU-20A5R degasser, an SPD-30AD DAD detector and the LabSolutions 5.54 SP5 software (Shimadzu, Japan). The separations were carried out using an Aqua C18, 250 × 4.6 mm, 5 µm column

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(Phenomenex, USA). The temozolomide samples were manufactured by PRI.

Analytical procedures

The method for the determination of the temozolomide chemical purity was as follows: eluent A: 0.5% acetic acid in water, eluent B: acetonitrile, gradient elution program: t (min)/%B: 0/8; 8/8; 28/40; 31/8; 36/8, column temperature: 25°C; autosampler temperature: 5 ± 2°C; flow rate: 1 mL/min; injection volume: 20 µL; UV detection wavelength: λ = 254 nm; diluent: water with 0.5% HAc : ACN (4 : 1, v/v); concentration of temozolomide in the investigated sample solutions: 1 mg/mL. The validation of the method for the determination of the temozolomide chemical purity as well as important notes on the sample preparation were described in the previous publication (2). The reference method for the temozolomide assay was as follows: mobile phase: 0.5% HAc in water : MeOH (9 : 1, v/v); isocratic elution; duration of analysis: 20 min; column temperature: 25°C; autosampler temperature: 5 ± 2°C; flow rate: 1 mL/min; injection volume: 10 µL; UV detection wavelength: λ = 254 nm; diluent: 0.5% HAc : ACN (9 : 1, v/v); concentration of temozolomide in the investigated sample solutions: 0.25 mg/mL.

The applied i-DReC settings were as follows: threshold: 500 mAU; wavelength for correction: 500 mAU (Auto); direction: +; intensity to extract: 500 mAU.

RESULTS AND DISCUSSION

The chromatographic procedure used for the determination of related substances in temozolomide is not suitable for the determination of the temozolomide assay. The concentration of TZ in the samples used for the determination of related substances is relatively high (1 mg/mL), so the TZ sig-

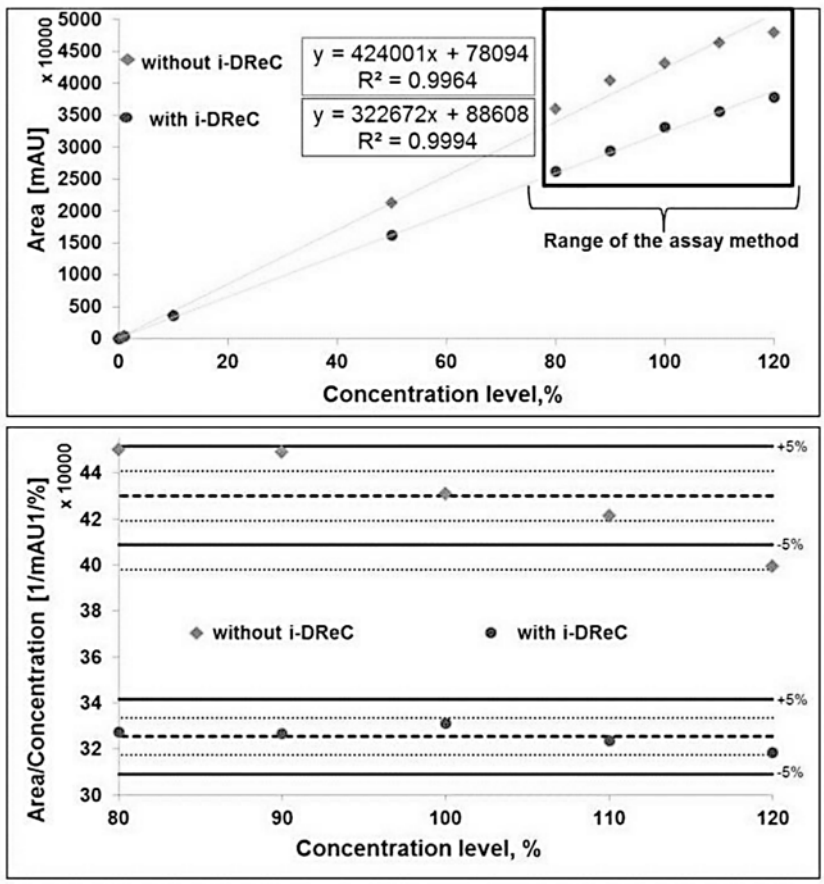

Figure 1. Comparison of calibration curves with application of i-DReC function (circles) and without application of i-DReC (squares) and comparison between the ratio of intensity/ concentration and concentration of temozolomide in the range used for assay determination (80–120% i.e., 0.8–1.2 mg/mL)

Table 1. Comparison of the results of temozolomide assay determined using the method for chemical purity with and without i-DReC and the reference assay method

	No. of sample	Reference assay method [%]	Assay determined using the method for chemical purity without i-DReC [%]	Assay determined using the method for chemical purity with i-DReC [%]
	1	100.6	76.2	100.0
	2	99.0	75.3	98.9
	3	100.0	76.5	100.4
	4	99.1	75.2	98.7
	5	99.6	75.8	99.6
	6	99.7	75.7	99.4
	7	98.5	75.6	99.3
	8	98.6	76.0	99.8
Statistical analysis comparison of the results of temozolomide assay				
	$\bar{x}$	**99.4**	**75.8**	**99.5**
	SD	0.7	0.4	0.6
Snedecor`s F-test	***F***	–	0.37	0.61
$F = SD_2^2/SD_1^2$ $F_{cr(\alpha = 0.05,\ f = n-1)}$	$\boldsymbol{F_{cr} = 3.79}$	$F \le F_{cr}$ – the difference between the standard deviations is not significant		
Student's *t*-test	***t***	-	79.156	0.310
$t = \frac{\lvert \bar{x}_1 - \bar{x}_2 \rvert}{\sqrt{s_1^2 + s_2^2}} \cdot \sqrt{n}$ $t_{cr(\alpha = 0.05,\ f = 2n-2)}$	$\boldsymbol{t_{cr} = 2.149}$		$t >> t_{cr}$ – the difference between the average results is significant	$t \le t_{cr}$ – the difference between the average results is not significant
Statistical analysis comparison of the results of linearity				
n			13	13
Regression line			y = 424002x + 78094	y = 322672x + 88608
Standard deviation			SD_a = 7733 SD_b = 496533 SD_{xy} = 1324961	SD_a = 2385 SD_b = 153165 SD_{xy} = 408708
R^2			0.9964	0.9994

nal is saturated. Therefore, it was necessary to develop another procedure for the determination of the TZ assay to perform routine analyses of the TZ samples manufactured by PRI (2). As the i-DReC function allows to perform a quantitative analysis even outside the linear range of the method, an attempt to determine the TZ assay with the use of the method destined for the determination of the TZ purity was undertaken. Calibration curves were plotted in the range from 0.30 µg/mL (0.03% in relation to the initial TZ concentration) to 1.2 mg/mL (120% in relation to the initial TZ concentration). Thus, the chosen calibration range covered the concentrations used for the determination of related substances according to the guidelines (8), i.e., from the limit of quantitation, which was formerly set at 0.30 µg/mL (0.03% in relation to the initial TZ concentration) level (2) to 120% of the specification limit for both unknown and known impurities, i.e., 1.2 µg/mL and 1.5 µg/mL, respectively (0.12% and 0.15% in relation to the initial TZ concentration, respectively). The calibration curve also contained the concentration range required for the assay determination, i.e., 80–120% of the test concentration (0.8–1.2 mg/mL).

The results of the temozolomide assay determination with and without the use of the i-DReC function were compared with the results obtained with the reference assay method (Table 1). The tests were carried out for eight independent temozolomide samples. As it is shown in Table 1, the assay of temozolomide is significantly underestimated when the calibration curve without the i-

DReC correction is used for the calculation of the results. On the other hand, the results calculated with the use of the calibration curve with the i-DReC correction are consistent, compared to the results acquired with the reference assay method. The performed statistical tests (Snedecor's F-test and Student's *t*-test) confirmed that the application of the i-DReC procedure allows to obtain valid results of the temozolomide assay in contrast to the procedure without the application of the i-DReC. The comparison of the calibration curves plotted for the results obtained with and without the application of the i-DReC clearly denotes that the linearity is lost at higher TZ concentration levels (Fig. 1). The application of the i-DReC function allowed to correct the linearity and carry out quantitative measurements even at high TZ concentrations. The comparison between the ratio of intensity/concentration and the concentration of temozolomide in the range used for the assay determination (80–120% i.e., 0.8–1.2 mg/mL) shows that the application of the i-DReC function allows to maintain the ratio within the ± 5% confidence level.

As the i-DReC is a relatively new function, there is a scarcity of literature data referring to this feature's application. However, there are two technical notes released by Shimadzu where the application of the i-DReC in pharmaceutical analyses is presented (6, 7). It was demonstrated that it is possible to simultaneously quantify both the major component at a high concentration level and the impurities at low levels with the application of the i-DReC. The results described in the current paper also confirm that the use of the i-DReC function allows to quantify the major component in pharmaceutical samples, even at high concentrations.

CONCLUSION

The comparison of the determination results of the temozolomide assay with the use of the analytical procedure for the purity determination and the application of the i-DReC function with the results obtained for the separate reference assay method revealed a good coherence between them. It is thus possible to use only one chromatographic run instead of two procedures to quantify both the major component and related substances, which allows to reduce the solvent consumption, lower the costs of the analysis and save time.

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