RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF TEMOZOLOMIDE AND *(S)* - PERILLYL ALCOHOL IN NANOPARTICULATE DOSAGE FORM

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ABSTRACT

A quick reverse-phase high-performance liquid chromatography (RP-HPLC) approach for the quantitative measurement of temozolomide (TMZ) and *(s)* - perillyl alcohol [(*S*)-POH] in a nanoparticulate system was developed and validated in the current work. The RP-HPLC method for the simultaneous estimation of TMZ and *(S)*-POH was developed using Agilent (Infinity 1260) HPLC system and Zorbax-C18 (4.6 x 150 mm i.d., 5µ; Agilent) as stationary phase. The optimized mobile phase comprised of ACN: water: MeOH (42:12:46 V/V/V; 42:08:50 V/V/V and 20:30:50 V/V/V) pumped at a flow rate of 0.8 mL min^{-1}, 0.8 mL min^{-1} and 1 mL min^{-1}, respectively. Drug separation was accomplished in an isocratic mode, and a PDA detector operating at 210 nm was used to track elution. The procedure was validated in accordance with ICH-Q2R1 standards. The responses of TMZ and (*S*)- POH were found to be linear at 50-175 µg mL^{-1} (ACN: water: MeOH 42:12:46 V/V/V and 42:08:50 V/V/V) and 50-175 µg mL^{-1} (ACN: water: MeOH 20:30:50 V/V/V) respectively. The percent recovery was determined to be between 97% and 103%, demonstrating that the method's accuracy was adequate. The precision study's percent relative standard deviation (% RSD) was less than 2, indicating the accuracy of the suggested procedure. It was discovered that the established method for the quantitative determination of TMZ and (*S*)- POH in bulk and in hollow gold nanoparticles was accurate, precise, and specific. The developed technique can be applied to TMZ and (*S*)- POH routine testing and quality control in bulk and nanoparticulate systems.

Keywords: RP-HPLC, temozolomide, *(s)* - perillyl alcohol, hollow gold nanoparticles, ICH guidelines Q2R1

INTRODUCTION

Chemically, 4-methyl-5-oxo-2,3,4,6,8-pentazabicyclo [4.3.0]nona-2,7,9-triene-9-carboxamide[1] is the name of the anti-cancer medication temozolomide (TMZ). The literature review identified several techniques for estimating TMZ in bulk, dosage forms, and human plasma, including LC-MS, RP-HPLC, HPTLC, stability-indicating HPLC, and UV spectrophotometric approaches[2]. *(s)*- perillyl alcohol (*S*)-POH is an anti-cancer drug used in various formulations to treat glioblastoma[3]. It is independent of MGMT expression. In addition to this, it suppresses Ras/Raf/ERK and mTOR pathways with an increase in Bak and reduction in Bcl-xL, cyclin A, B1 and cdk2 levels[4,5]. It is also responsible for the sensitization of glioma cells to TMZ therapy through induction of GRP78 and CHOP expression. Various reports on clinical trials i.e; phase I and II, with or without temozolomide, have shown promising effects even in patients with recurrent glioblastomas[3]. Chemically, it is (4-prop-1-en-2-ylcyclohexen-1-yl) methanol[6]. RP-HPLC, LC-MS, and other bioanalytical techniques are documented for the analysis of (*S*)- POH in dosage forms[7]. Fig. 1 represents the chemical structures of TMZ and (*S*)- POH.

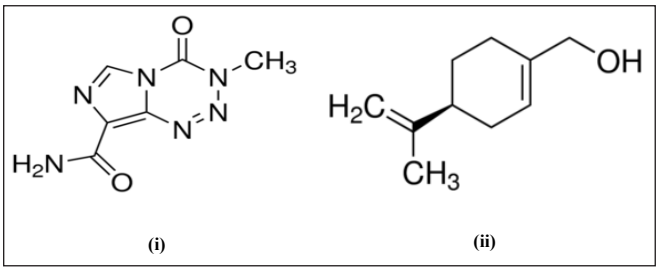

Fig. 1: Molecular structure of (i) temozolomide and (ii) *(s)* - perillyl alcohol

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Our lab is working on the development of hollow gold nanoparticles for the treatment of glioblastoma. Currently, there is no developed RP-HPLC method for concurrently estimating TMZ and (*S*)-POH in the pharmaceutical dosage form. There is no marketed formulation of TMZ and (*S*)-POH (hollow gold nanoparticle or any other pharmaceutical dosage form) for glioblastoma therapy. Literature states that TMZ and (*S*)- POH in the ratio of 1:1, when used in combination or if conjugated together, can successfully manage the remission of this deadly cancer disease[3,7]. Therefore, a fixed-dose combination hollow gold nanoparticle formulation containing a combination of TMZ and (*S*)- POH or its conjugate was developed to treat the tumour. The goal of the current work was to create an RP-HPLC method that would be simple, reliable, and precise for quantifying TMZ and (*S*)- POH in PEGylated hollow gold nanoparticles.

MATERIALS AND METHODS

Temozolomide (TMZ) and (*s*) - perillyl alcohol ((*S*)-POH) were procured from Sun Pharma Pvt. Ltd., Mumbai, India and Sigma Aldrich, Mumbai, India, respectively. Methanol and HPLC grade acetonitrile were purchased from S.D. Fine Chemical Ltd., Mumbai, India. Dilutions and solution preparations were carried out using Millipore water. Analytical grade products were used for all other compounds and reagents.

HPLC instrumentation and chromatographic conditions

An agilent HPLC system (Infinity 1260) with an autosampler and a photodiode array (PDA) detector was used to develop analytical methods. At a temperature of 28 °C ± 0.02 °C, TMZ and (*S*)- POH were separated by chromatography using an agilent Zorbax-C18 (4.6 x 150 mm, 5 µ particle size) column. ACN: water: MeOH (42:12:46 V/V/V; 42:08:50 V/V/V; 20:30:50 V/V/V) were the components of the mobile phase. For ACN: water: MeOH 42:12:46 V/V/V and 42:08:50 V/V/V, the flow rate was 0.8 mL min^{-1}, while for ACN: water: MeOH 20:30:50 V/V/V, it was 1 mL min^{-1}. At 210 nm, the analyte peaks were found using a 20 µL injection volume.

Preparation of standard stock solution

Standard stock solutions of both the drugs were prepared by individually dissolving 10 mg of TMZ and 10 mg of (*S*)- POH in 10 mL of methanol. These solutions were then sonicated to yield distinct concentrations of 1000 µg mL^{-1}.

Method development studies

To obtain 100 µg mL^{-1} of TMZ and 100 µg mL^{-1} of (*S*)- POH solution, aliquots of the standard stock solutions were further diluted using the mobile phase ACN: water: MeOH (42:12:46 V/V/V; 42:08:50 V/V/V; 20:30:50 V/V/V). Following the loading of the samples into the autosampler, chromatograms were recorded.

Validation of analytical method

System suitability

The purpose of the system appropriateness research is to confirm whether the chromatographic system is appropriate for the planned analysis. The compatibility of the approach with the system was confirmed using six injections of 100 µg mL^{-1} TMZ and (*S*)- POH, respectively. USP standards were followed in the recording of parameters such as tailing factor, theoretical plates, peak area, and resolution[8].

Specificity

Specificity is the ability to test the target analyte accurately and efficiently in the presence of additional chemicals/reagents that are expected to be present in the sample matrix. 500µL of a placebo sample (PEGylated hollow gold nanoparticles composed of excipients such as trisodium citrate, cobalt chloride, sodium borohydride, gold chloride, and thiol PEG amine, excluding both the drugs) was dissolved in 10 mL of a mobile phase, such as ACN: water: MeOH (42:12:46 V/V/V; 42:08:50 V/V/V; 20:30:50 V/V/V)[2].

Precision

Three levels of precision were examined: re-producibility, intermediate precision and repeatability.

Repeatability: The test concentration (TMZ 100 µg mL^{-1}: (*S*)- POH 100 µg mL^{-1}) was created for a minimum of six determinations (n=6), and the results were recorded at 210 nm. Percentage RSD, or relative standard deviation, was noted.

Intermediate Precision: There were two levels to this study: intra-day and inter-day. Preparing the test concentration (TMZ 100 µg mL^{-1} and (*S*)- POH 100 µg mL^{-1}) and assaying six times (n = 6) at three distinct time intervals resulted in intra-day precision. Three days during sample analysis (n = 6) were used to measure inter-day precision. Values for the percent relative standard deviation (% RSD) were computed[9,10].

Accuracy

A standard solution of TMZ and (*S*)- POH was spiked into the PEGylated hollow gold nanoparticle placebo sample. Trisodium citrate, cobalt chloride, sodium borohydride, and gold chloride were used to create a placebo sample of PEGylated hollow gold nanoparticles, which was then coated with thiol PEG amine (a PEGylation reagent). This batch of placebos was chosen for research on accuracy. A 500µL solution of a pre-analyzed placebo formulation was mixed with concentrations of TMZ and (*S*)- POH equal to 80%, 100%, and 120%. The research was conducted in triplicate, and the mean percent RSD was ascertained[8].

Linearity and range

The capacity of an analytical method to produce test findings that are exactly proportionate to the concentration (quantity) of analyte in the sample (within a specified range) is known as linearity. Usually, the variance of the slope or regression line is used to report it. A range is defined as the interval between the solute's highest and lower concentrations that have been shown to be linearly, precisely, and accurately determined. To verify the linearity of this approach, 50–175 µg mL^{-1} of TMZ and (*S*)- POH were extracted from the stock solution, and subjected to HPLC analysis. Three duplicates of each concentration measurement were made. Plotting peak regions against concentrations were performed, and linear regression analysis was accomplished[11].

LOD and LOQ

The method's sensitivity is determined by the limits of quantification (LOQ) and detection (LOD). While LOQ is the lowest measurable concentration, LOD is the lowest detectable concentration of the analyte by the technique. The point at which a measured value exceeds its associated uncertainty is known as the limit of determination, or LOD. The quantitation limit, a parameter of quantitative assays for low chemical concentrations in sample matrix structures, is particularly helpful for detecting contaminants or degradation products. The following formulae were used to derive LOD and LOQ from drug standard calibration curves[12].

$$\text{Limit of detection (LOD)} = \frac{3.3 \times \sigma}{\text{Slope}}$$

where σ is the standard deviation and slope of the calibration curve of the respective drug.

$$\text{Limit of quantification (LOQ)} = \frac{10 \times \sigma}{\text{Slope}}$$

Robustness

An analytical method's robustness is a measure of the extent to which it can withstand small but intentional changes in method parameters, and shows how reliable it is when used normally. By altering the chromatographic conditions, 100 µg mL^{-1} of each test concentration of TMZ and (*S*)- POH were administered. To assess the method's reliability, the following adjustments were made[11]. Three duplicates of the study were analyzed.

Variation in flow rate: To record the chromatographic responses, the flow rate was changed to 0.9 mL min^{-1} and 1.1 mL min^{-1} (ACN: water: MeOH 20:30:50 V/V/V) and 0.7 mL min^{-1} and 0.9 mL min^{-1} (ACN: water: MeOH 42:12:46 V/V/V and 42:08:50 V/V/V).

Temperature variation: For all three mobile phases, the temperature was changed to 27 °C and 29 °C to measure the chromatograms of TMZ and (*S*)- POH.

RESULTS AND DISCUSSION

Optimization of chromatographic parameters

The most appropriate mobile phase composition for reproducible peaks with symmetry within USP limits was found to be ACN: water: MeOH (42:12:46 V/V/V; 42:08:50 V/V/V and 20:30:50 V/V/V) at a flow rate of 0.8 mL min^{-1} (ACN: water: MeOH 42:12:46 V/V/V and 42:08:50 V/V/V) and 1 mL min^{-1} (ACN: water: MeOH 20:30:50 V/V/V). The Zorbax-C18 (4.6 x 150 mm, 5 µ, Agilent) column maintained at 28°C ± 0.02 °C was found to be optimal. Fig. 2 (a,b,c) shows the chromatogram of standard TMZ and (*S*)- POH (100 µg mL^{-1}).

System suitability

The resolution was determined to be greater than two, theoretical plates were not less than 2000, and system appropriateness characteristics such as tailing factor were found to be less than two. Each criterion was satisfied in accordance with the USP acceptance standards. The %RSD values for all the parameters were found to be less than 2 (Retention time-0.002; Resolution- 0.001; No. of theoretical plates- 0.001; Tailing factor- 0.002; Peak area- 0.004). The results of system suitability are expressed in Table I.

Specificity

Given that the mobile phase did not interfere during the retention period of any drug, it was determined that the newly developed methods were specific. At 210 nm, the percent interference was determined to be 0.001 AU. Fig. 3 displays a chromatogram that illustrates the method's specificity.

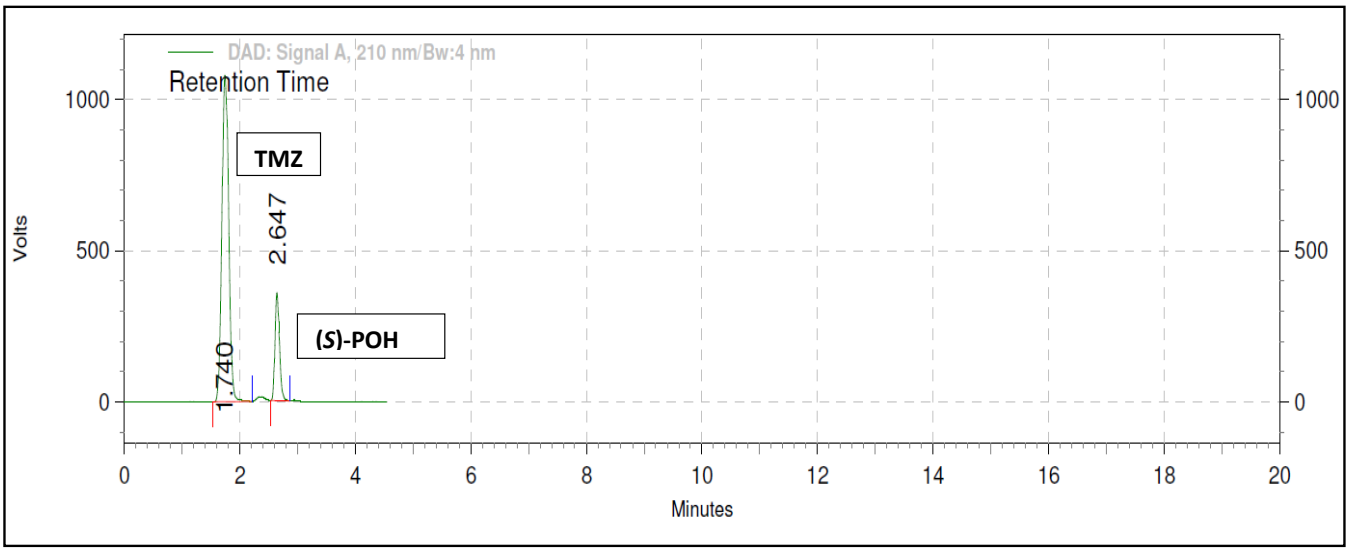


Fig. 2a: Chromatogram of standard TMZ and (*S*)-POH in optimized chromatographic conditions (ACN: water: MeOH 42:12:46 V/V/V, 0.8mL min^{-1} at 28 °C)

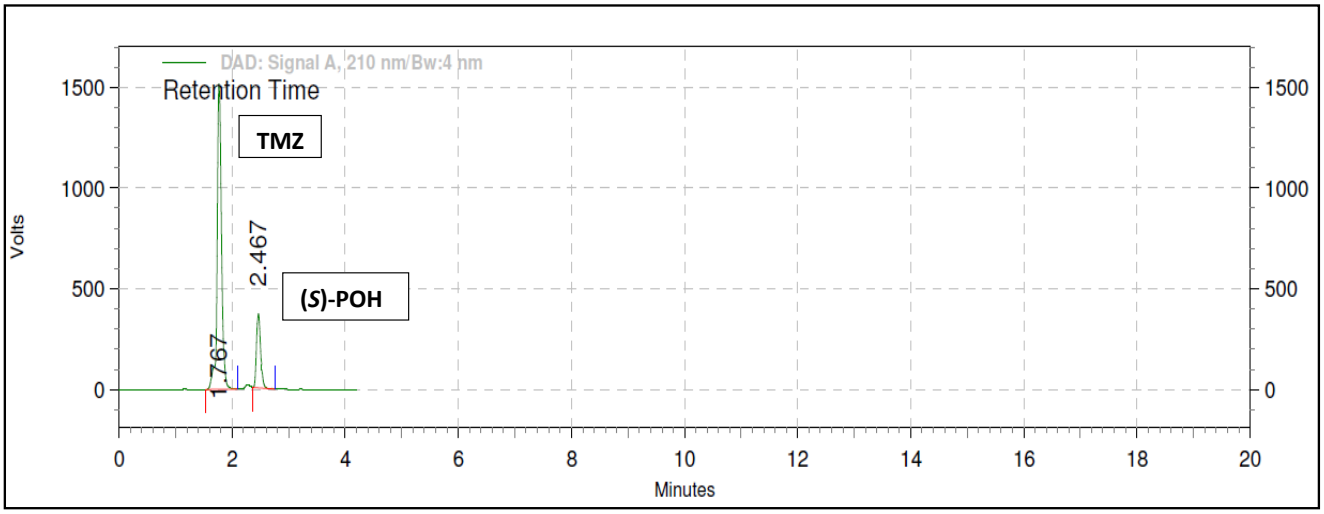


Fig. 2b: Chromatogram of standard TMZ and (*S*)-POH in optimized chromatographic conditions (ACN: water: MeOH 42:08:50 V/V/V, 0.8mL min^{-1} at 28°C)

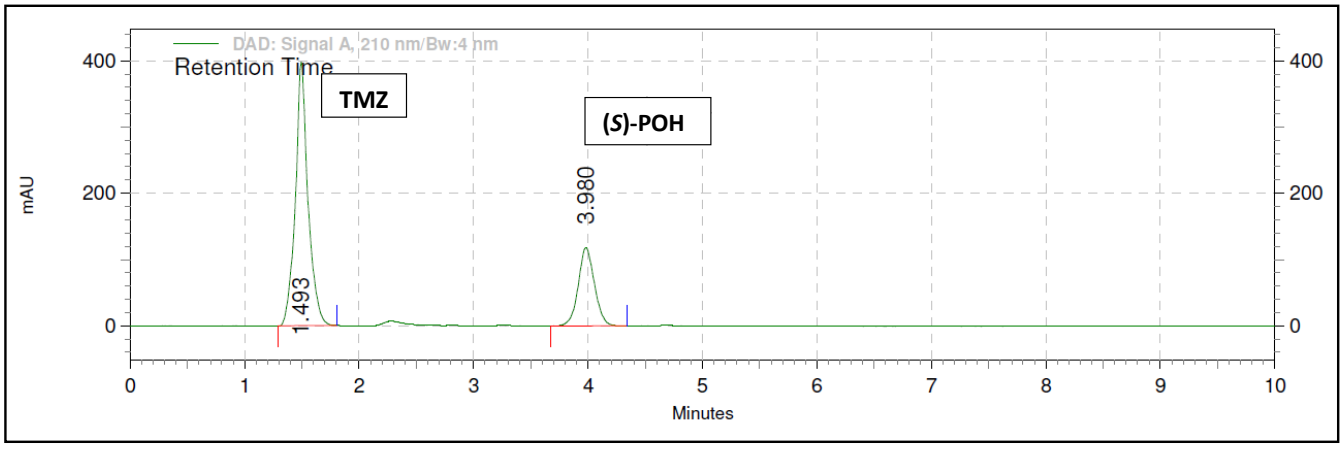


Fig. 2c: Chromatogram of standard TMZ and (*S*)-POH in optimized chromatographic conditions (ACN: water: MeOH 20:30:50 V/V/V, 1mL min^{-1} at 28°C)

Table I: System suitability study of TMZ and (*S*)-POH

Parameter	ACN: water: MeOH 42:12:46 V/V/V, 0.8mL min^{-1}, 28°C * ± SD		ACN: water: MeOH 42:08:50 V/V/V, 0.8mL min^{-1}, 28°C *± SD		ACN: water: MeOH 20:30:50 V/V/V, 1mL min^{-1}, 28°C *± SD		Acceptance criteria
	TMZ	(*S*)-POH	TMZ	(*S*)-POH	TMZ	(*S*)-POH	
Retention time (min.)	1.740±0.02	2.647±0.01	1.76±0.01	2.46±0.02	1.493±0.02	3.980±0.01	--
Resolution	5.09±0.01		5.27±0.01		11.3±0.01		Rs > 2
No. of theoretical plates	2475±0.03	5486±0.02	2895±0.01	5417±0.01	2435±0.02	3818±0.03	N>2000
Tailing factor	1.13±0.02	1.19±0.01	0.9±0.03	1.19±0.02	0.9±0.03	1.06±0.02	T<1.5
Peak area	18669608 ± 312	4066338 ± 3245	16562331 ±212	3915287 ± 3678	13252070 ± 454	4806612 ± 2398	--

** The data is shown as Mean ± SD, *n: 6 -number of injections, *SD: standard deviation*

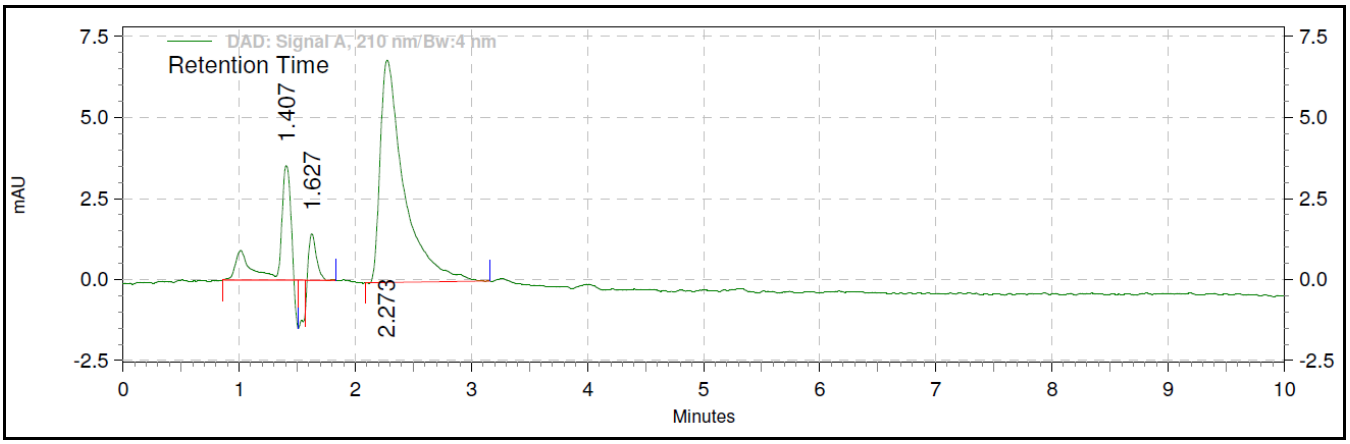
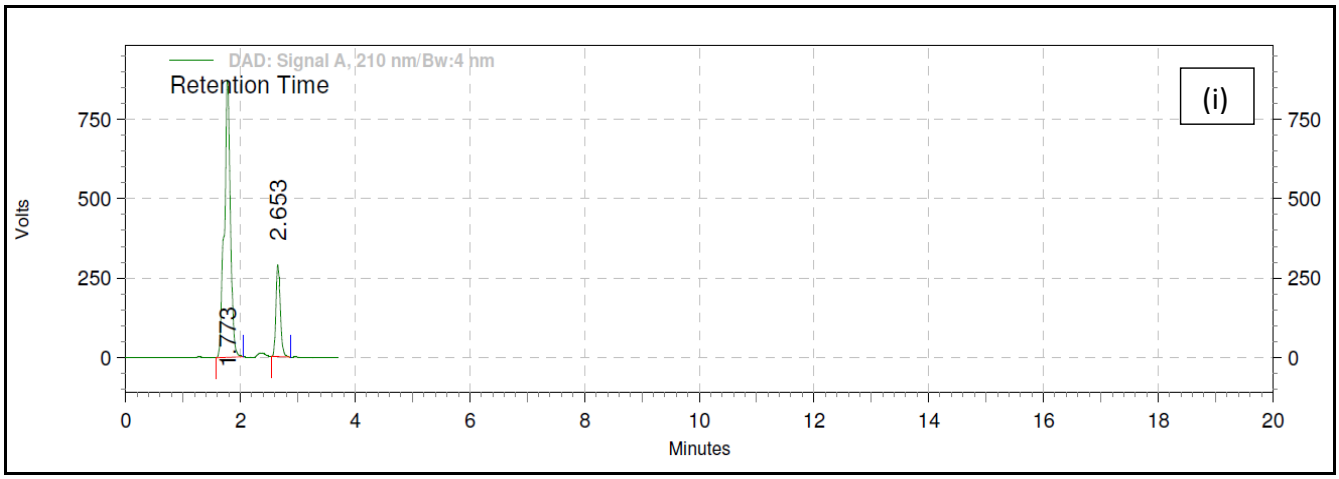


Fig. 3: HPLC chromatogram of placebo batch (PEGylated hollow gold nanoparticles dissolved in mobile phase)



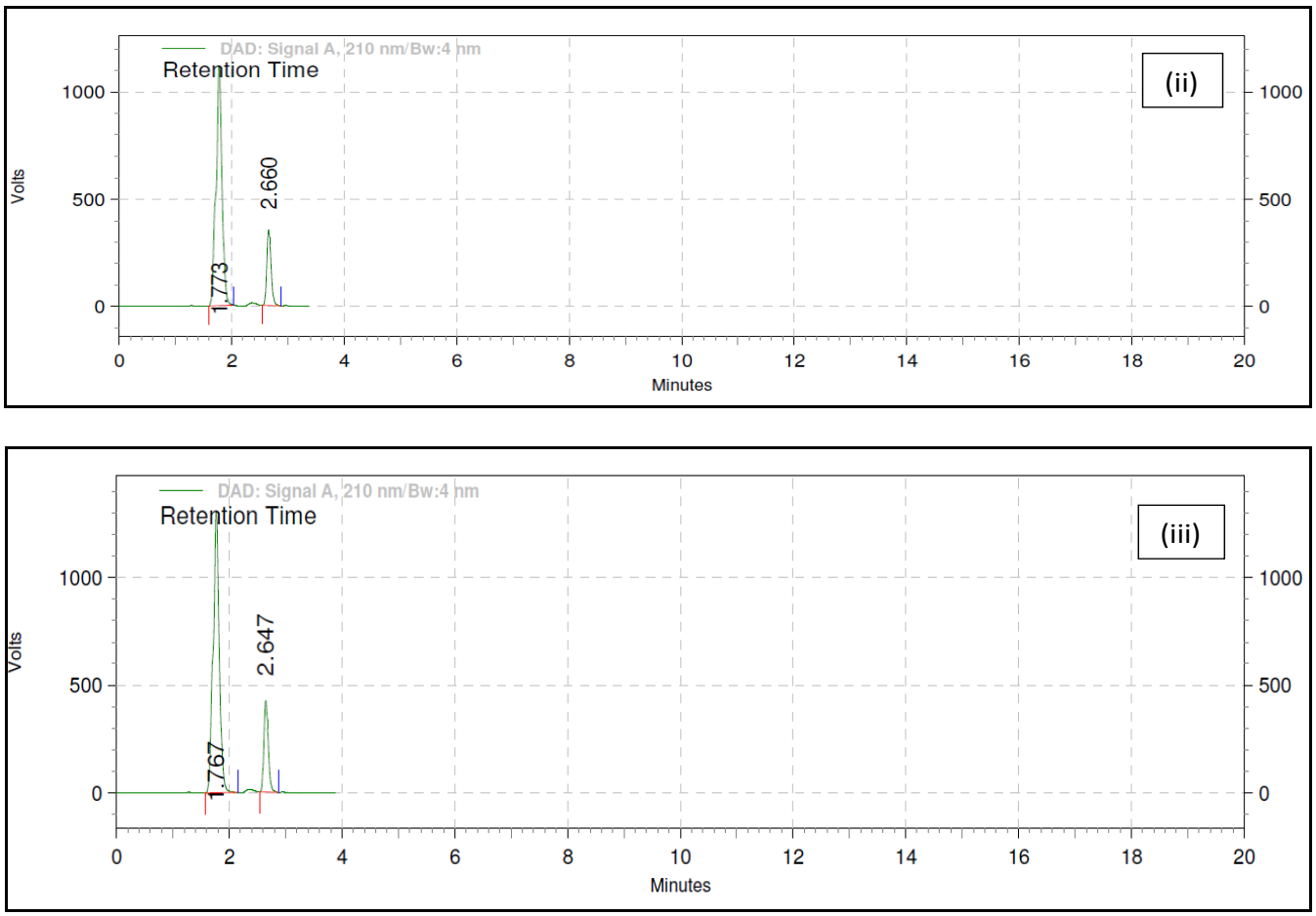
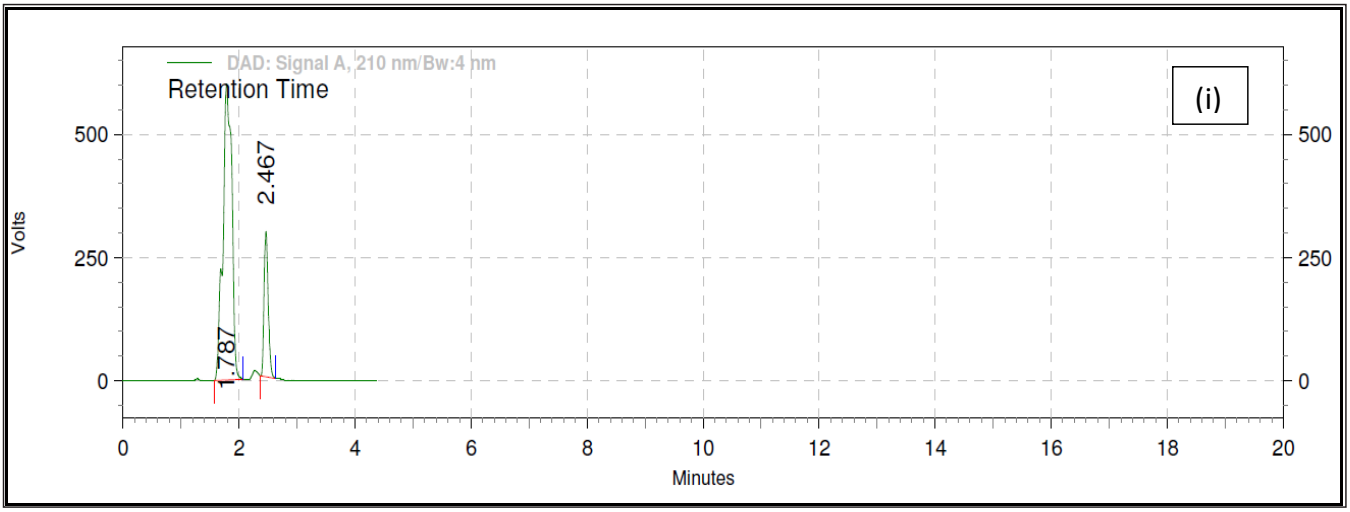


Fig. 4a: Chromatogram of TMZ and (*S*)-POH in accuracy study at (i) Level 1: 80%, (ii) Level 2: 100% and (iii) Level 3: 120% for ACN: water: MeOH 42:12:46 V/V/V



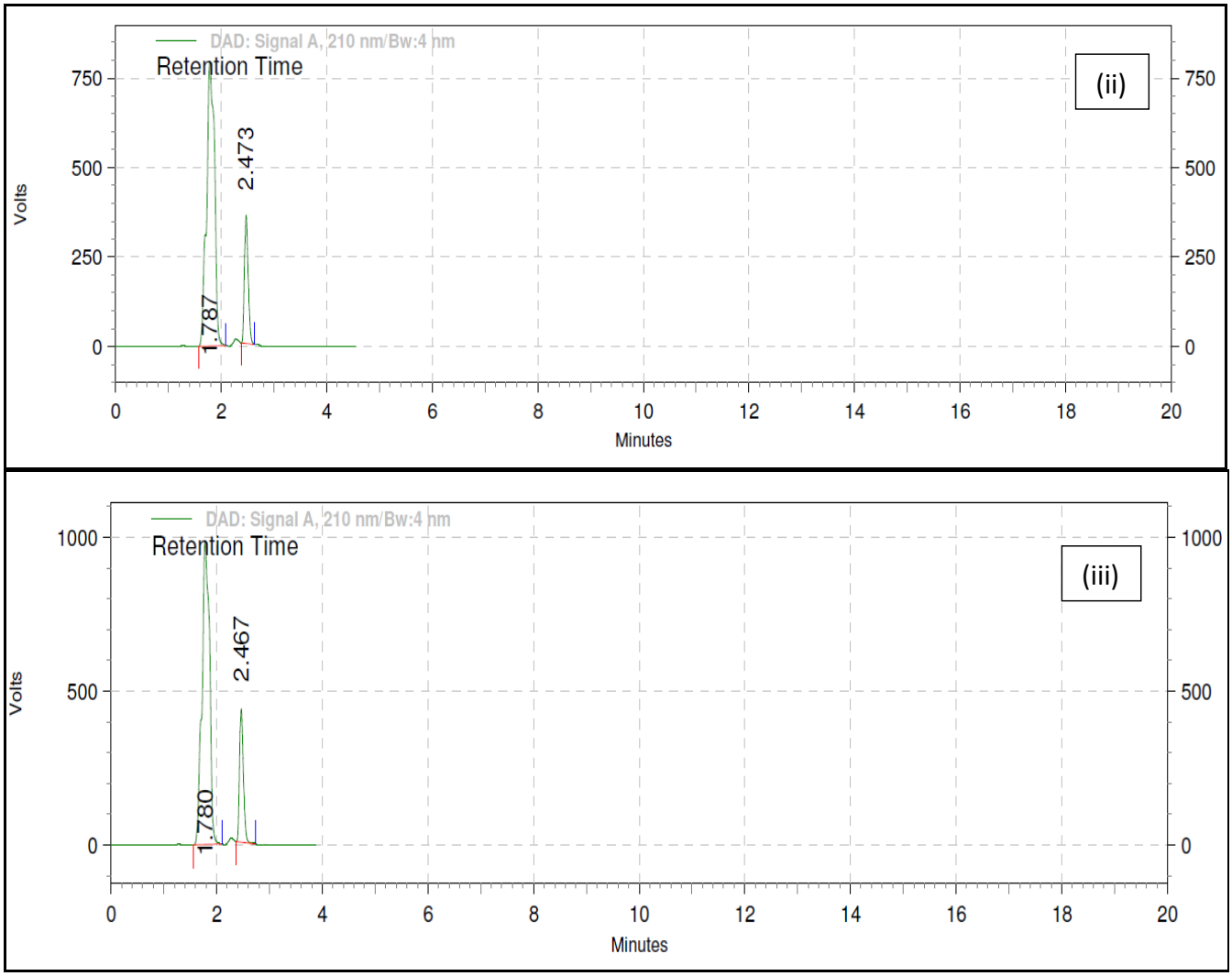
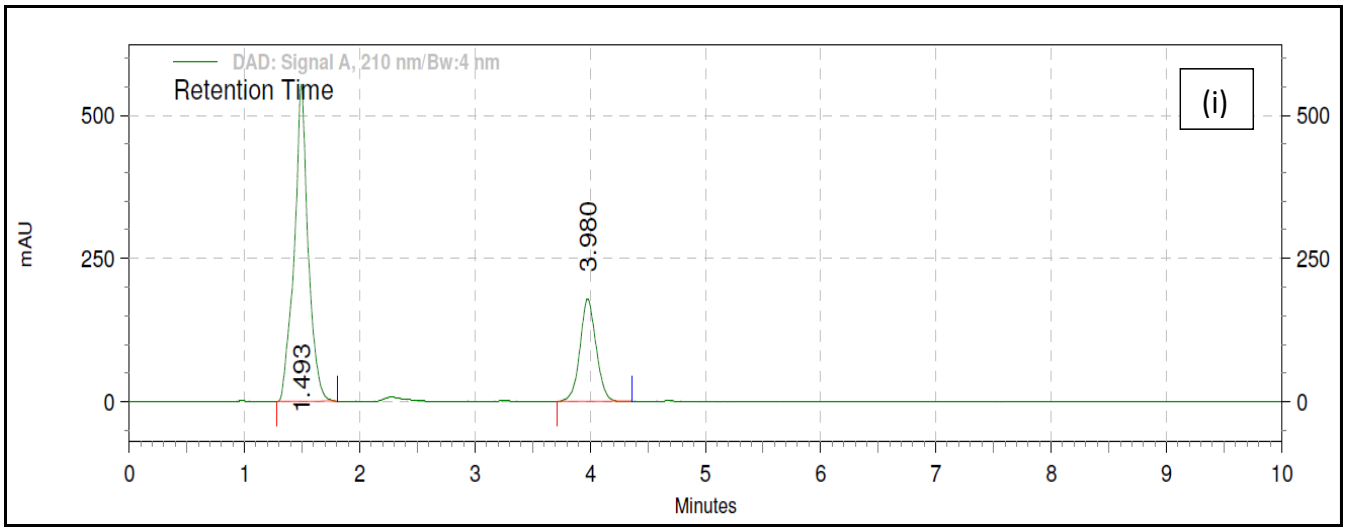


Fig. 4b: Chromatogram of TMZ and (*S*)-POH in accuracy study at (i) Level 1: 80%, (ii) Level 2: 100% and (iii) Level 3: 120% for ACN: water: MeOH 42:08:50 V/V/V



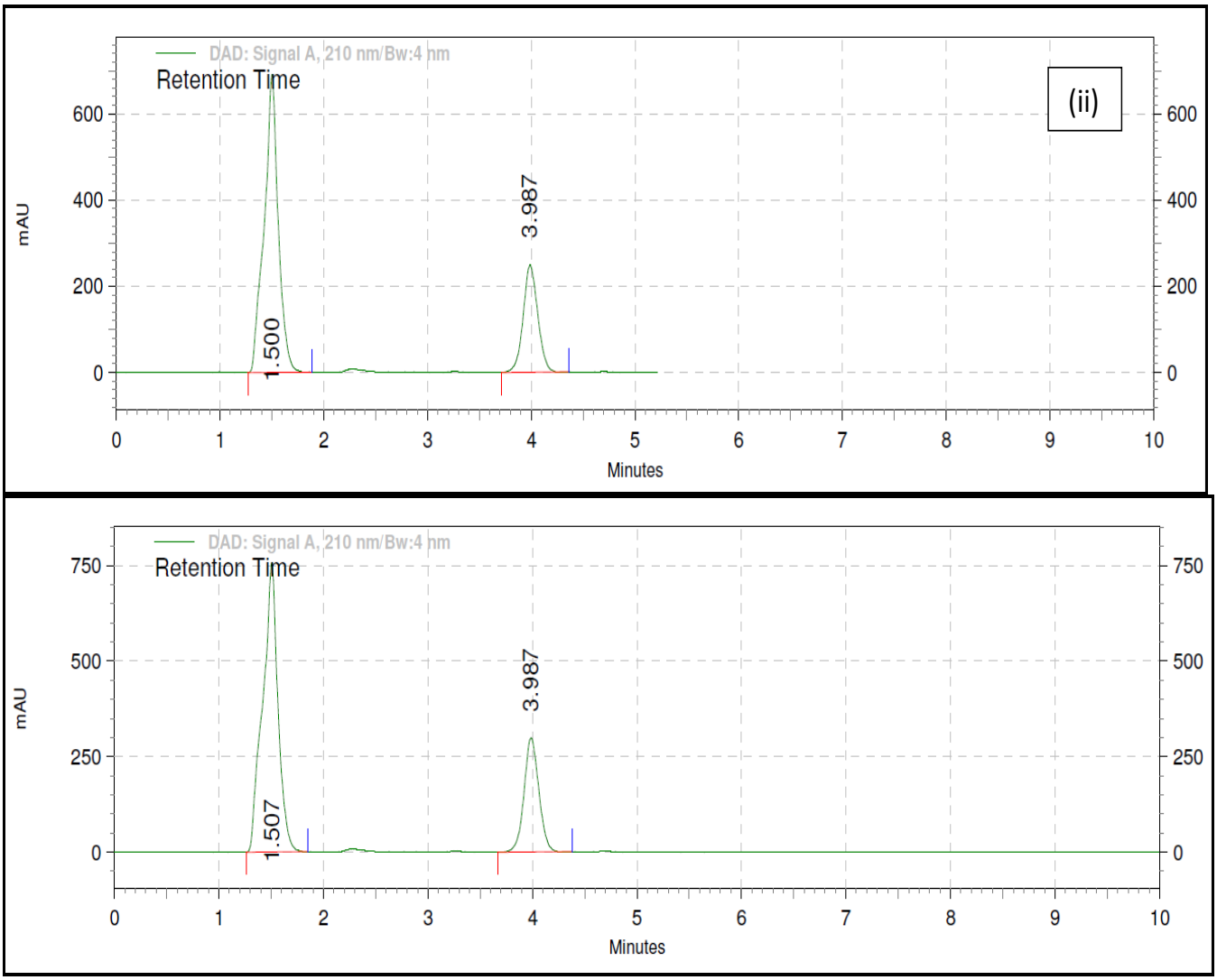


Fig. 4c: Chromatogram of TMZ and (*S*)-POH POH in accuracy study at (i) Level 1: 80%, (ii) Level 2: 100% and (iii) Level 3: 120% for ACN: water: MeOH 20:30:50 V/V/V

Precision

For TMZ and (*S*)-POH, repeatability, intraday precision, and interday precision studies were conducted, and it was discovered that the percentage RSD values were less than 2% for both drugs. The developed procedure was discovered to be accurate. Table II presents the precision study's findings.

Accuracy

Three distinct limits were used to calculate the mean recovery of TMZ and (*S*)- POH. At all the three levels, the percentage recovery was determined to be between 97 % and 103%, and the percentage RSD was less than 2. The results obtained at 80%, 100%, and 120% were found to be beyond the predetermined parameters. It ultimately contributed to the method's accuracy being determined. Table III presents the accuracy study's results, and Fig. 4(a,b,c) illustrates the chromatograms.

Linearity and range

Six dilutions of the working standard solutions 100µg mL^{-1} of TMZ and (*S*)-POH in the range of 50-175 µg mL^{-1} were generated to verify the linearity of the method. Three injections (n=3) of each dilution were made into the column. For both the drugs, the established method's correlation coefficients (R^2 values) were found to be under certain limits (more than 0.99). Fig. 5 illustrates linearity curves, while Fig. 6 depicts the linearity chromatogram (i, ii, iii).

Table II: Precision study of TMZ and (*S*)-POH

Precision parameter		Peak area ± SD					
		(ACN: water: MeOH 42:12:46 V/V/V)* ±SD		(ACN: water: MeOH 42:08:50 V/V/V)*±SD		(ACN: water: MeOH 20:30:50 V/V/V)*±SD	
		TMZ	(*S*)-POH	TMZ	(*S*)-POH	TMZ	(*S*)-POH
Repeata-bility	Peak area	18675438 ± 59694	4056280 ± 11301	16612270 ±94910	3971320 ±18508	13288318 ±4222	4823736 ±5329
	% RSD	0.319	0.278	0.571	0.466	0.031	0.11
Intraday study (n=6)	Time 1	1860226	4046121	16451018	3954347	13253174	4840535
	Time 2	1863601	4045729	16596084	3957531	13238798	4848056
	Time 3	1861105	4060241	16526421	3966214	13246998	4843717
	mean ± SD	1861644 ± 1751	4050697 ± 8268	16524508 ±72552	3959364 ±6142	13246323 ±7211	4844103 ±3775
	% RSD	0.094	0.204	0.439	0.15	0.054	0.077
Interday study (n=6)	Day 1	1860790	4091805	16548216	3960004	13258309	4821977
	Day 2	1855142	4077808	16280348	3950543	13271808	4823425
	Day 3	1859613	4077348	16352429	3951050	13259613	4829015
	mean ± SD	1858515 ± 2979	4082320 ± 8217	16393664 ±138613	3953866 ±5321	13263243 ±7446	4824806 ±3716
	% RSD	0.160	0.201	0.845	0.134	0.056	0.077

**The data is shown as Mean ± SD, *n: 6- number of injections, *SD: standard deviation, *%RSD: percentage relative standard deviation*

Table III: Accuracy study of TMZ and (*S*)-POH

Drug	Level	Amount added (mg)	Amount found*± SD (mg)	%Recovery*± SD	%RSD*
ACN: water: MeOH 42:12:46 V/V/V					
TMZ	80%	80	80.19±0.16	100.24 ± 0.21	0.211
	100%	100	99.98±0.04	99.98 ± 0.04	0.045
	120%	120	120.01±0.45	100.01 ± 0.37	0.376
(*S*)-POH	80%	80	80.05±0.06	100.07 ± 0.08	0.084
	100%	100	100.16±0.15	100.16 ± 0.15	0.151
	120%	120	120.29±0.26	100.24 ± 0.21	0.217
ACN: water: MeOH 42:08:50 V/V/V					
TMZ	80%	80	79.88±0.31	99.85±0.38	0.389
	100%	100	100.44±0.38	100.44±0.38	0.382
	120%	120	120.07±0.46	100.06±0.38	0.384

	Level	Amount added (mg)	Amount found*± SD (mg)	%Recovery*± SD	%RSD*
(*S*)-POH	80%	80	80.19±0.42	100.24±0.53	0.089
	100%	100	99.91±0.08	99.91±0.08	0.089
	120%	120	119.97±0.24	99.98±0.20	0.205
ACN: water: MeOH 20:30:50 V/V/V					
TMZ	80%	80	80.04±0.06	100.05±0.07	0.076
	100%	100	100.04±0.08	100.04±0.08	0.081
	120%	120	120.01±0.01	100.01±0.01	0.016
(*S*)-POH	80%	80	80.01±0.03	100.02±0.04	0.049
	100%	100	100.01±0.01	100.01±0.01	0.013
	120%	120	120.00±0.02	100.00±0.02	0.021

**The data is shown as Mean ± SD, *n: 3- number of injections, *SD: standard deviation, *%RSD: percentage relative standard deviation*

Table IV: Robustness study of TMZ and (*S*)-POH

		TMZ		(*S*)-POH	
Variable	**Level (±) (-1,+1)**	**Mean Retention time (min)* ± SD**	**% RSD***	**Mean Retention time (min)* ± SD**	**% RSD***
ACN: water: MeOH 42:12:46 V/V/V					
Flow rate (mL min^{-1})	0.7 mL	1.75±0.0005	0.033	2.65±0.0005	0.021
	0.9 mL	1.73±0.0005	0.033	2.63±0.0005	0.0217
Temperature (°C)	27 °C	1.74±0.0005	0.033	2.64±0.0005	0.021
	29 °C	1.741±0.0005	0.033	2.641±0.001	0.037
ACN: water: MeOH 42:08:50 V/V/V					
Flow rate (mL min^{-1})	0.7 mL	1.78±0.0005	0.032	2.46±0.0005	0.023
	0.9 mL	1.76±0.0005	0.032	2.44±0.0005	0.023
Temperature (°C)	27 °C	1.77±0.0005	0.033	2.45±0.0005	0.023
	29 °C	1.771±0.0005	0.033	2.451±0.001	0.04
ACN: water: MeOH 20:30:50 V/V/V					
Flow rate (mL min^{-1})	0.9 mL	1.491±0.001	0.06	3.981±0.001	0.025
	1.1 mL	1.491±0.0005	0.038	3.982±0.0005	0.014
Temperature (°C)	27 °C	1.491±0.0005	0.038	3.98±0.0005	0.014
	29 °C	1.491±0.0005	0.038	3.9811±0.0005	0.01

**The data is represented as Mean ± SD, *n: 3- number of injections at each level, *SD: standard deviation, *%RSD: percentage relative standard deviation*

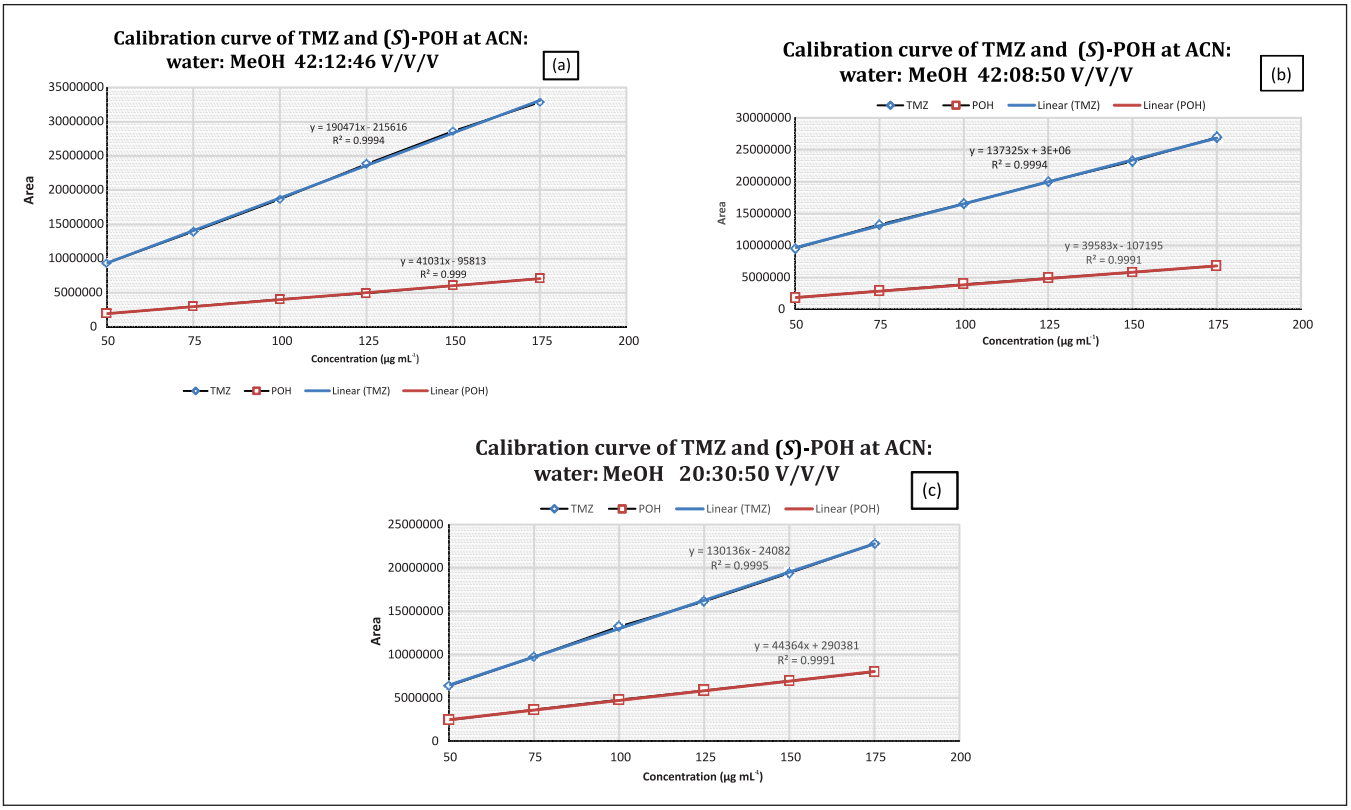
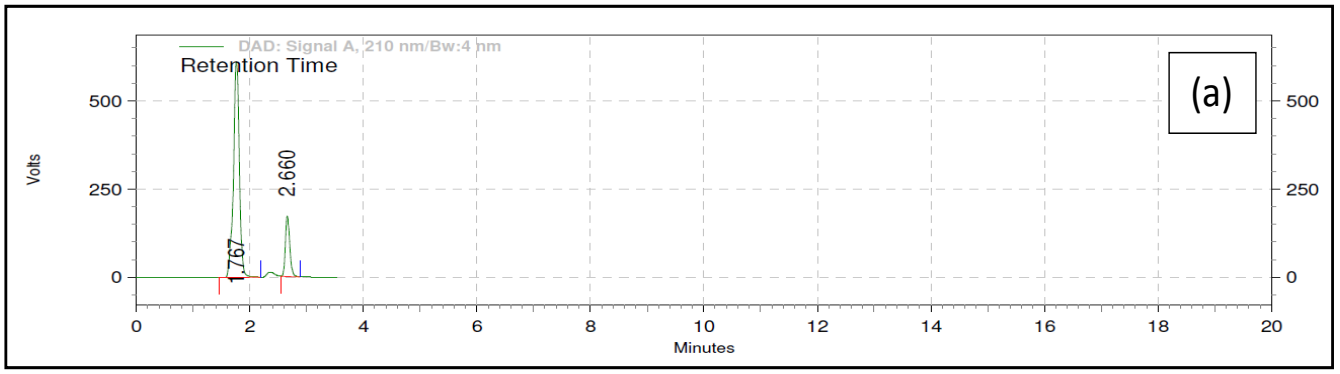
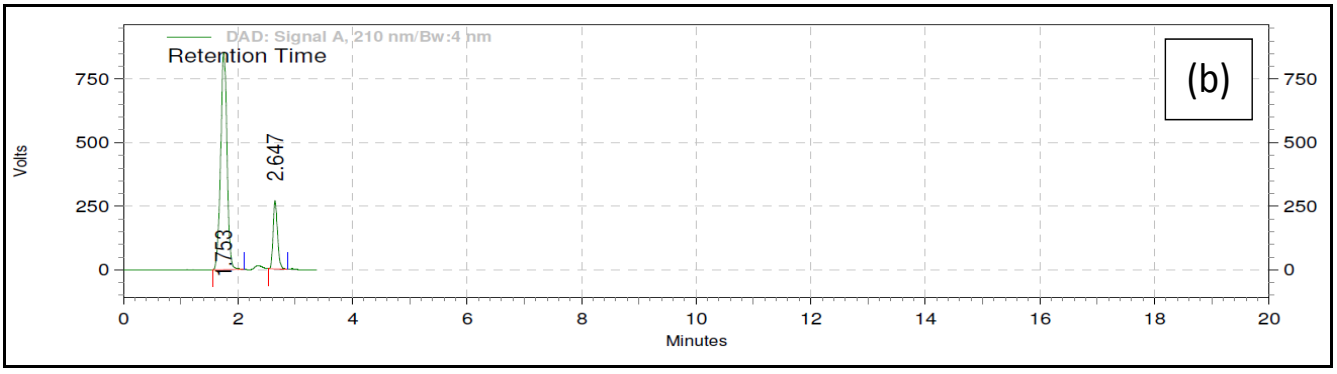


Fig. 5: Calibration curve of TMZ and (*S*)-POH at ACN: water: MeOH (a) 42:12:46 V/V/V (b) 42:08:50 V/V/V and (c) 20:30:50 V/V/V

*(*n: 3- number of injections of each concentration. The data is represented in the graph as Mean ± SD, *SD: standard deviation)*





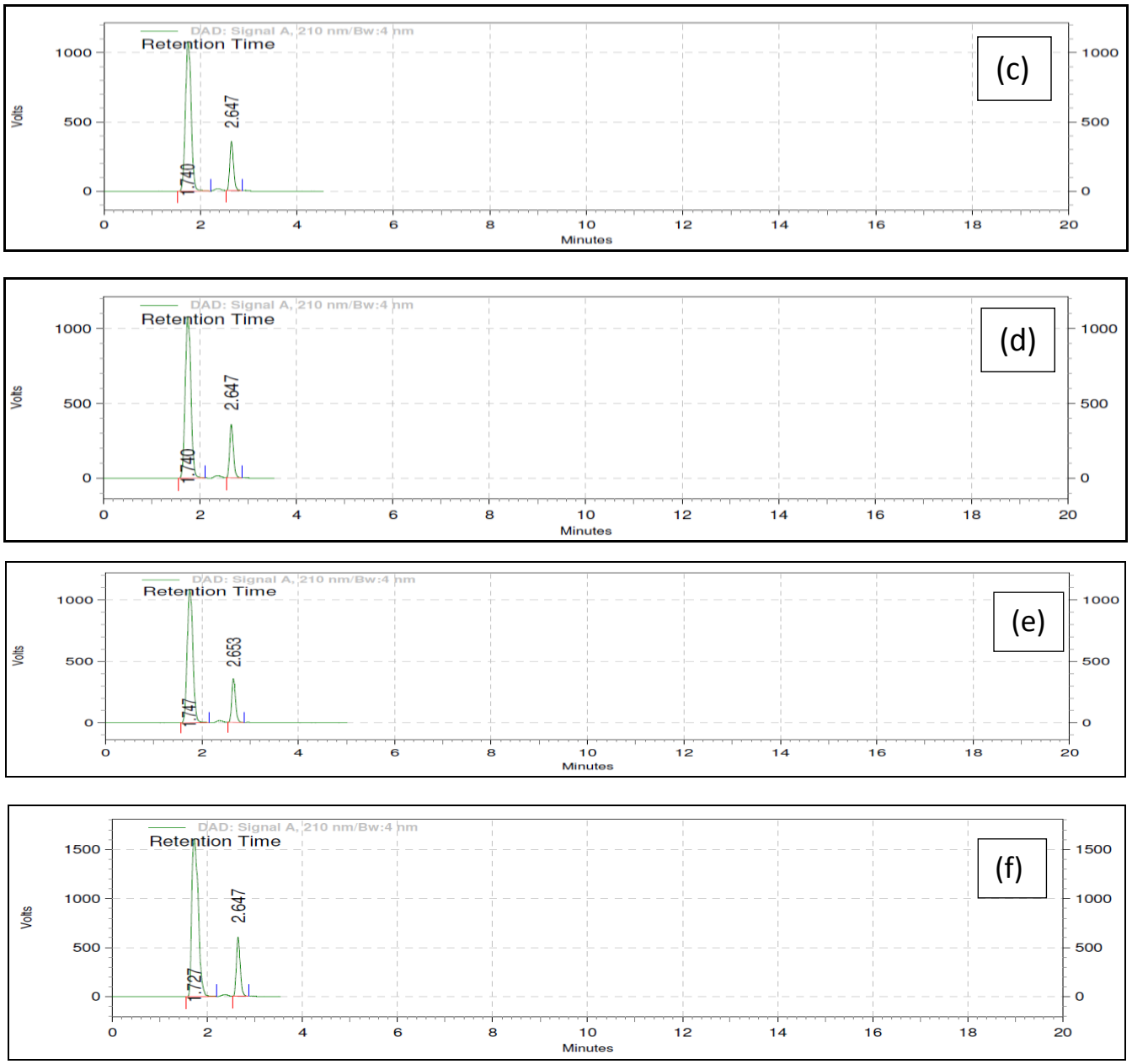
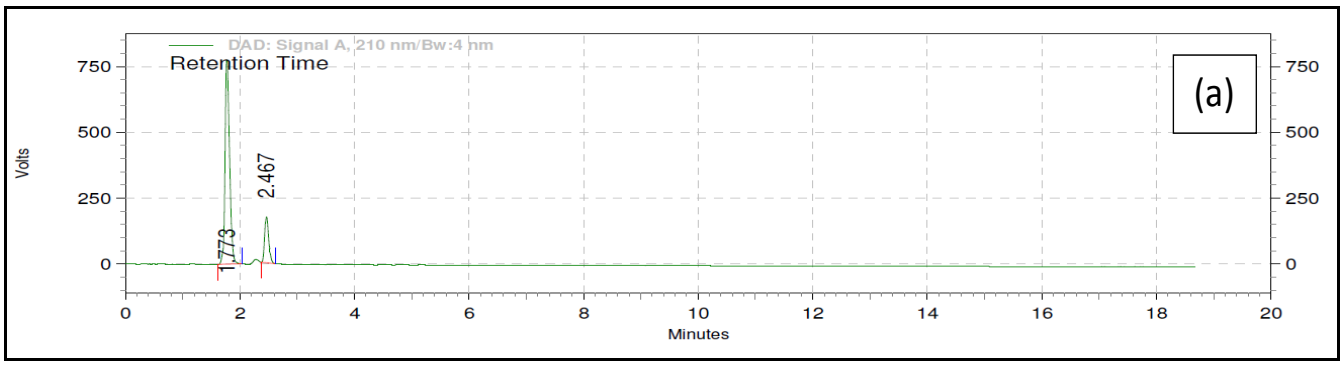


Fig. 6i: HPLC chromatograms of TMZ and (*S*)-POH at (a) 50 µg mL^{-1} – (f) 175 µg mL^{-1} concentrations, respectively for ACN: water: MeOH 42:12:46 V/V/V



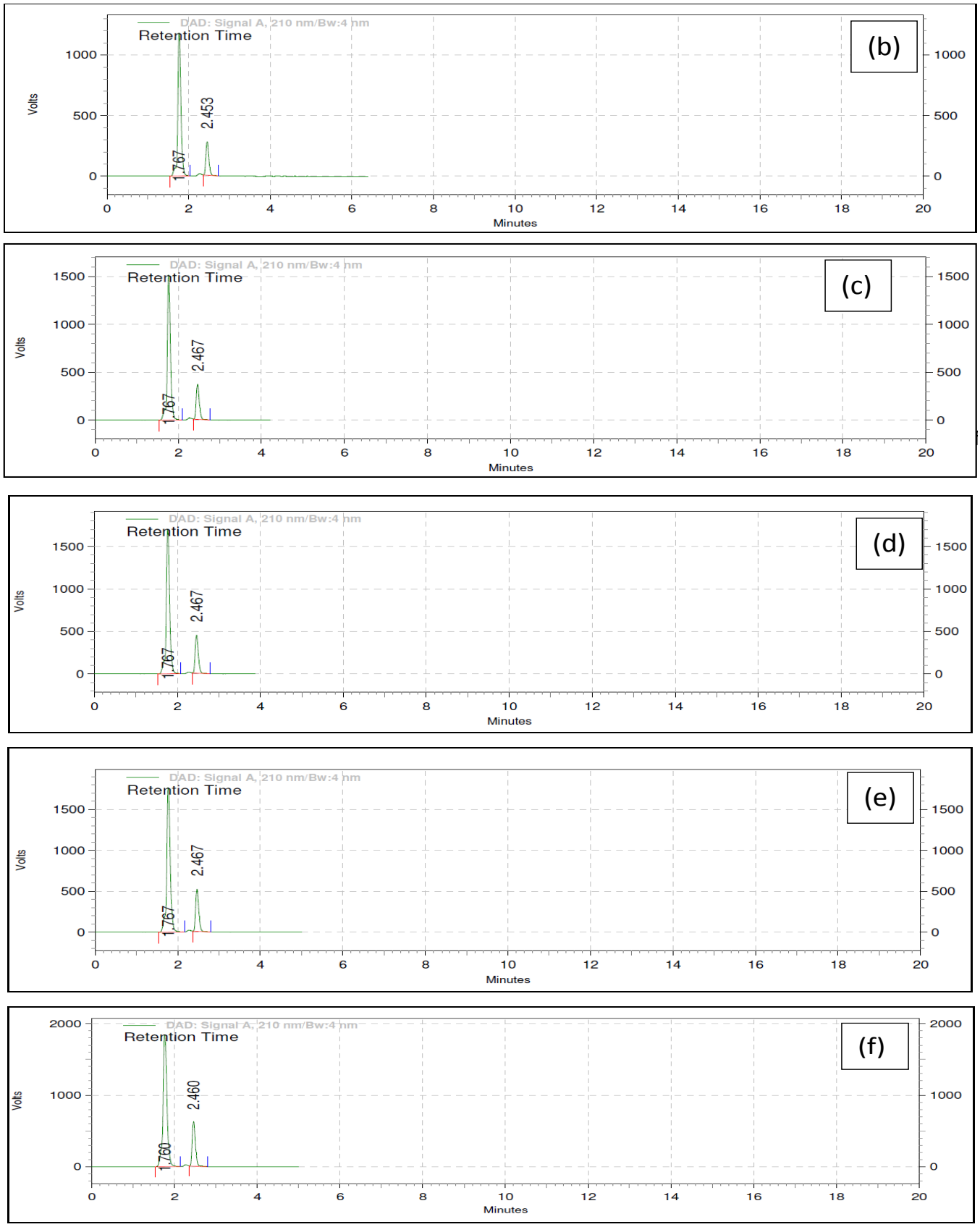
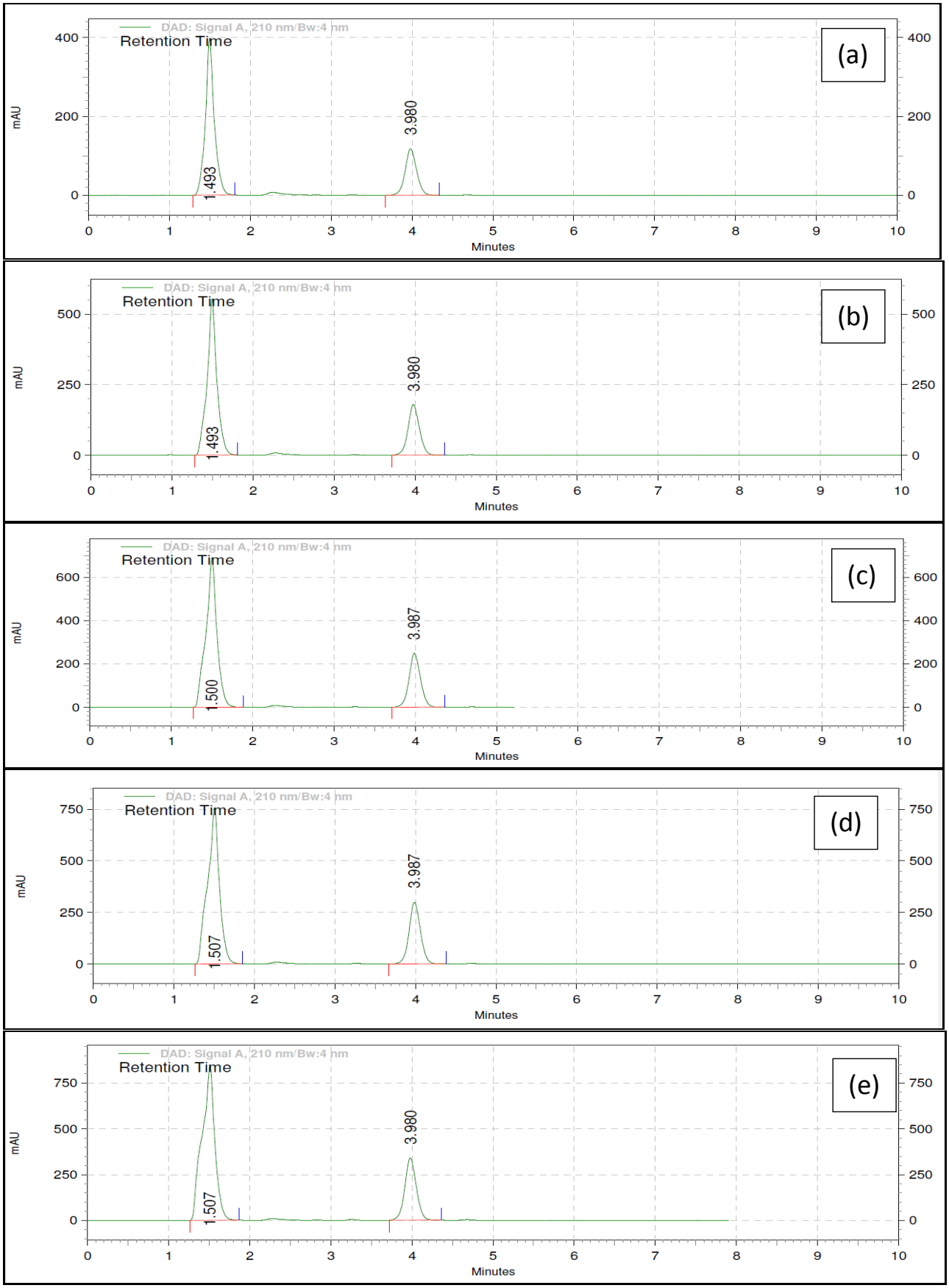


Fig. 6ii: HPLC chromatograms of TMZ and (*S*)-POH at (a) 50 µg mL^{-1}– (f) 175 µg mL^{-1} concentrations, for ACN: water: MeOH 42:08:50 V/V/V


DAD: Signal A, 210 nm/Bw:4 nm
Retention Time
(a)
mAU
1.493
3.980
Minutes
DAD: Signal A, 210 nm/Bw:4 nm
Retention Time
(b)
mAU
1.493
3.980
Minutes
DAD: Signal A, 210 nm/Bw:4 nm
Retention Time
(c)
mAU
1.500
3.987
Minutes
DAD: Signal A, 210 nm/Bw:4 nm
Retention Time
(d)
mAU
1.507
3.987
Minutes
DAD: Signal A, 210 nm/Bw:4 nm
Retention Time
(e)
mAU
1.507
3.980
Minutes

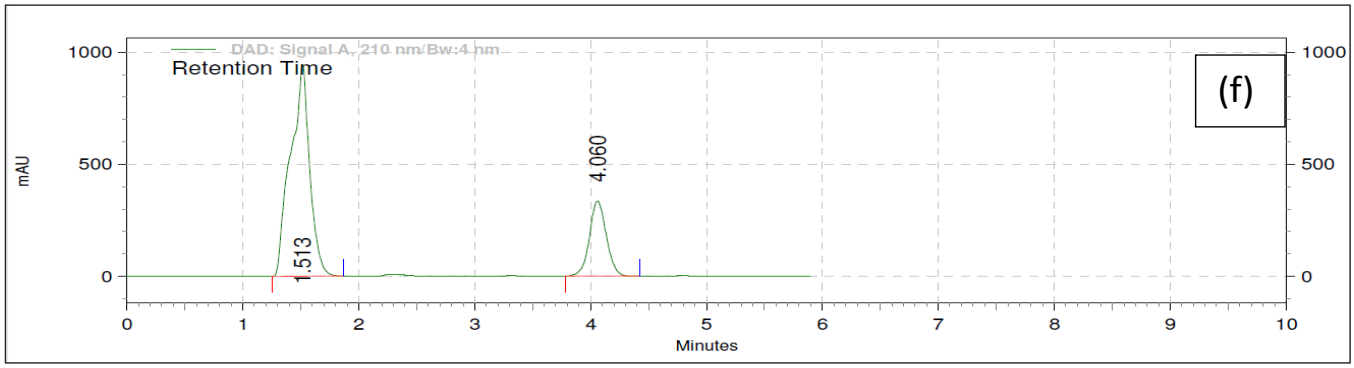


Fig. 6iii: HPLC chromatograms of TMZ and (*S*)-POH at (a) 50 µg mL^{-1} – (f) 175 µg mL^{-1} concentrations, for ACN: water: MeOH 20:30:50 V/V/V

LOD and LOQ

LOD and LOQ were estimated based on the regression equation's slope and peak response. The LODs of TMZ and (*S*)- POH were found to be 0.051 µg mL^{-1} & 0.66 µg mL^{-1}, respectively, at ACN: water: MeOH 42:12:46 V/V/V; 1.4 µg mL^{-1} & 0.5 µg mL^{-1} at ACN: water: MeOH 42:08:50 V/V/V and 0.188 µg mL^{-1} & 0.276 µg mL^{-1} at ACN: water: MeOH 20:30:50 V/V/V. The LOQs of TMZ and (*S*)- POH were found to be 0.156 µg mL^{-1} & 2 µg mL^{-1}, respectively at ACN: water: MeOH 42:12:46 V/V/V; 4.24 µg mL^{-1} & 1.66 µg mL^{-1} at ACN: water: MeOH 42:08:50 V/V/V and 0.572 µg mL^{-1} & 0.837 µg mL^{-1} at ACN: water: MeOH 20:30:50 V/V/V.

Robustness

Since minor changes in the chromatographic conditions had no effect on the peak area, the method was considered robust. After analyzing the data, it has been demonstrated that the method is not greatly impacted by variations in temperature or flow rate. As a result, even when these two parameters were changed, the designed approach remained stable. The robustness study's findings are displayed in Table IV and were within the acceptable limits.

DISCUSSION

An innovative, effective, and dependable RP-HPLC method has been created for the quantitative determination of TMZ and (*S*)- POH in the PEGylated hollow gold nanoparticle dosage form in accordance with ICH specifications. According to USP/ICH norms, the validation acceptance criteria were fulfilled in every instance. The use of this method in the estimation of TMZ and (*S*)- POH in nanoparticulate dosage forms revealed that the excipients did not affect the calculations. Thus, this approach was distinctive. The sample recoveries in the formulation were consistent, which suggested that the excipients in the formulation did not affect the estimate. The aforementioned observations' findings demonstrate how basic, accurate, and more specific RP-HPLC method was designed for the quantitative determination of TMZ and (*S*)- POH in the nano formulation. Furthermore, an array of analytical methods for determining TMZ and (*S*)- POH alone and in conjunction with other medications have been reported. It has been established that the special approach presented in this paper is the first of its type. RP-HPLC is not yet accessible, nevertheless, for the simultaneous measurement of TMZ and (*S*)- POH in conjunction with drug release analysis from nanoparticulate dose forms. Because the developed method only needs a minimal amount of solvent and has a short chromatographic time, it is easy to implement.

CONCLUSION

To quantitatively estimate TMZ and (*S*)- POH in the nanoparticulate dose form, a simple, reproducible, and accurate RP-HPLC analytical method was established. The created procedure demonstrated the capacity to use the RP-HPLC technology to separate TMZ and (*S*)- POH when combined in a dosage form. In accordance with ICH criteria Q2R1, it was efficiently validated. Taking this into account, it can be said that, depending on its bioavailability at the site of action, this developed RP-HPLC analytical method can be utilized for quantitative analysis of pharmaceutical dosage forms with TMZ and (*S*)- POH with fixed dose combinations and other drugs for the treatment of glioblastoma, lung cancer, breast cancer, urinary bladder cancer, etc. For both *in vitro* and *in vivo* release studies, the ideal formulation of PEGylated hollow gold nanoparticles containing TMZ and (*S*)- POH was chosen.

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REFERENCES

1. National Center for Biotechnology Information. PubChem Compound Summary for CID 5394, Temozolomide. https://pubchem.ncbi.nlm.nih.gov/compound/Temozolomide. Accessed Mar. 8, 2023.
2. Michels L.R., Fachel F.N.S., Azambuja J.H., Gelsleichter N.E., Braganhol E. and Teixeira H.F.: HPLC–UV method for temozolomide determination in complex biological matrices: Application for *in vitro*, *ex vivo* and *in vivo* studies. **Biomed. Chromatogr.,** 2019, 33(10), 1-10.
3. Yeruva L., Pierre K.J., Elegbede A., Wang R.C. and Carper S.W.: Perillyl alcohol and perillic acid induced cell cycle arrest and apoptosis in non small cell lung cancer cells. **Cancer Lett.,** 2007, 257(2), 216-226.
4. Cho H.Y., Swenson S. and Thein T.Z.: Pharmacokinetic properties of the temozolomide perillyl alcohol conjugate (NEO212) in mice, **Neuro-oncology Adv.,** 2020, 2(1), 160.
5. Hua H.Y., Zhao Y.X., Liu L., Ye Q.X. and Ge S.W.: High-performance liquid chromatographic and pharmacokinetic analyses of an intravenous submicron emulsion of perillyl alcohol in rats, **J. Pharm. Biomed. Anal.,** 2008, 48(4), 1201-1205.
6. National Center for Biotechnology Information. PubChem Compound Summary for CID 10819, Perillyl alcohol. https://pubchem.ncbi.nlm.nih.gov/compound/Perillyl-alcohol. Accessed Mar. 8, 2023.
7. Chen T.C., da Fonseca C.O., Levin D. and Schönthal A.H.: The monoterpenoid perillyl alcohol: Anticancer agent and medium to overcome biological barriers, **Pharmaceutics,** 2021, 13(12), 2167.
8. Gupta A., Stratton S.P. and Myrdal P.B.: An HPLC method for quantitation of perillyl alcohol in a topical pharmaceutical cream formulation, **J. Pharm. Biomed. Anal.,** 2005, 37(3), 447-452.
9. Row K.H., Lee C.H. and Kang J.H.: Parameter estimation of perillyl alcohol in RP-HPLC by moment analysis, **Biotechnol. Bioprocess Eng.,** 2002, 7(1), 16-20.
10. Gupta A. and Myrdal P.B.: Development of a perillyl alcohol topical cream formulation, **Int. J. Pharm.,** 2004, 269(2), 373-383.
11. Bhadra S., Das S.C., Roy S., Arefeen S. and Rouf A.S.S.: Development and validation of RP-HPLC method for quantitative estimation of vinpocetine in pure and pharmaceutical dosage forms, **Chromatogr. Res. Int.,** 2011, 1-7.
12. Kowalska M., Woźniak M., Kijek M., Mitrosz P., Szakiel J. and Turek P.: Management of validation of HPLC method for determination of acetylsalicylic acid impurities in a new pharmaceutical product, **Sci. Rep.**, 2022, 12(1), 1.