Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

Stability Indicating Uplc Method for Quantitative Estimation of Gadobutrol in Gadobutrol Solution for Intravenous Administration

Ramya Sudha Pemmada, Bharath Ponnuri, Ramachandran Dittakavi*

Department of Chemistry, University College of Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.

(Received: 07 October 2023 Revised: 12 November 2023 Accepted: 06 December 2023)

KEYWORDS
Gadobutrol, UPLC, Degradation, Validation, Stability indicating.

ABSTRACT:

A simple and rapid stability-indicating, reversed phase ultra-performance liquid chromatography (UPLC) method was developed for the quantitative estimation of Gadobutrol in Gadobutrol solution for intravenous administration. The separation was achieved on Acquity UPLC CSH Phenyl-Hexyl (150 x 2.1mm, 1.7µm), in mobile phase consisted of pH 3.8 ammonium acetate buffer and acetonitrile in the ratio of (85:15 volume/volume). The flow rate was maintained at 0.5 mL/ min. UV detection was carried out at 195 nm. Column oven temperature was maintained 40°C and sample cooler temperature 5°C, injection volume 2 µL and run time was 6 minutes. The retention time of Gadobutrol was noted to be 3.1 minutes respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

1. INTRODUCTION

Gadobutrol (Gd-DO3A-butrol) is a gadolinium-based MRI contrast agent (GBCA). It received marketing approval in Canada and the United States [1-4]. As of 2007, it was the only GBCA approved at 1.0 molar concentrations [5]. Gadobutrol is marketed by Bayer AG as Gadovist, and by Bayer HealthCare Pharmaceuticals as Gadavist [6]. In India, it is also marketed by Vivere Imaging as Viv-butrol [7].

Gadobutrol [8] solution for injection is the complex consisting of gadolinium (III) and the macro cyclic dihydroxy-hydroxymethylpropyl-tetraazacyclododecane-triacetic acid (butrol), and is an injectable neutral contrast medium for magnetic resonance imaging (MRI). Gadobutrol is to be administered by intravenous injection. The chemical name of Gadobutrol is 10-[(1SR,2RS)-2,3-dihydroxy-1-hydroxymethylpropyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid, gadolinium complex. Corresponding to the molecular formula $C_{18}H_{31}GdN_4O_9$. It has a relative molecular mass of 604.72 g/mol. Gadobutrol solution is a sterile, clear, colorless to pale yellow solution containing 604.72 mg gadobutrol per mL (equivalent to 1 mmol/mL).

The literature review reveals that there are no UPLC methods were statemented in major pharmacopoeias like USP, EP, JP and BP. Only few analytical methods were reported till date for the estimation of Gadobutrol by using high performance liquid chromatography (HPLC) [9].

Hence we tried to develop stability indicating the UPLC technique for Gadobutrol in gadobutrol solution for intravenous administration. The present work describes a simple, stability indicating UPLC method for the determination of Gadobutrol in gadobutrol solution for intravenous administration according to ICH guidelines [10].

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

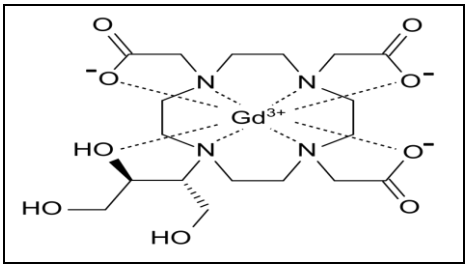


Figure 1. Chemical structure of Gadobutrol

2. MATERIALS AND METHODS

Materials

Analytical-grade Ammonium acetate, Glacial acetic acid, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide and water, reagents and chemicals were procured from Merck Chemicals. Mumbai, India. Gadobutrol API standard was procured from Pharmaffiliates, India. Gadobutrol (Gadovist 1.0 mmol/ml) were obtained from local market (Manufactured: Bayer Zydus Pharma Private Limited) and Tamilnadu India.

Instrumentation

Waters-Acquity H Class equipped with Empower[3] software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model).

Method

Preparation of 0.025M ammonium acetate buffer

1.9142 g of ammonium acetate was accurately weighed and transferred into 1000 mL beaker, 900 mL of HPLC grade water was added and dissolved, then the pH of the solution was adjusted to 3.8 with glacial acetic acid and made up to volume with water. The solution was filtered through 0.22µm membrane filter.

Preparation of mobile phase

Prepared a mixture of 850 mL of pH 3.8 ammonium acetate buffer and 150 mL of acetonitrile in the ratio of 85:15 (%volume/volume). The solution was filtered through 0.22µm membrane filter.

Preparation of diluent

Mobile phase is used as a diluent.

Preparation of standard solution

Accurately weighed 20.18 mg of Gadobutrol working standard was transferred into a 20 mL volumetric flask. 10 mL of diluent was added and sonicated to dissolve. The solution was diluted to volume with diluent and mixed well.

Preparation of sample solution

Accurately transferred 4.0 mL of the sample into 250 mL volumetric flask, added 120 mL of diluent and shaken for 5 minutes to mixed well and dilute to volume with diluent and mix well. Further transferred 5 mL of the resulting solution into 50 mL volumetric flask and dilute to volume with diluent and mixed well.

Preparation of placebo solution

Accurately transferred 4.0 mL of the placebo solution into 250 mL volumetric flask, added 120 mL of diluent and shaken for 5 minutes to mixed well and dilute to volume with diluent and mix well. Further transferred 5 mL of the resulting solution into 50 mL volumetric flask and dilute to volume with diluent and mixed well.

Chromatographic conditions

Chromatographic analysis was performed on Waters Acquity UPLC CSH Phenyl-Hexyl (150 x 2.1mm, 1.7µm) column. The mobile phase consisted of pH 3.8 ammonium acetate buffer and methanol in the ratio of 85:15 v/v. The flow rate was 0.5 mL/min, column oven temperature 40°C, sample cooler temperature was maintained 5°C the injection volume was 2 µL, and detection was performed at 195 nm using a photodiode array detector (PDA) and run time was 6 minutes.

3. METHOD DEVELOPMENT

Spectroscopic analysis of compound Gadobutrol showed that maximum UV absorbance (λmax) at 195 nm respectively. To develop a suitable and robust UPLC method for the determination of Gadobutrol,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

different mobile phases were employed to achieve the best separation and resolution. The method development was started with Acquity UPLC CSH Phenyl-Hexyl 150 x 2.1mm, 1.7µm column with the following different mobile phase compositions like that 0.1% OPA pH 1.8 and acetonitrile in the ratio of 95:5 v/v. 0.1% OPA pH adjusted to 3.0 with triethylamine and acetonitrile in the ratio of 90:10 v/v. Acetate Buffer pH 3.2 and acetonitrile in the ratio of 98:2 v/v. It was observed that when Gadobutrol was injected, higher retention time, Peak Tailing, not satisfactory.

For next trial the mobile phase composition was change same but pH of the acetate buffer slightly increased 3.2 to 3.8. The mobile phase composition was acetate buffer pH 3.8 and acetonitrile in the ratio of 85:15 v/v. respectively as eluent at flow rate 0.5 mL/min. UV detection as performed at 195 nm. The retention time of Gadobutrol is 3.1 minutes and the peak shape was good. The chromatogram of Gadobutrol standard using the proposed method is shown in **Figure 4** system suitability results of the method are presented in **Table 1.**

4. RESULTS AND DISCUSSION

The developed RP-UPLC method extensively validated for assay of Gadobutrol using the following parameters.

4.1 Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **Figure 2** showed no peak at the retention time of Gadobutrol peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Gadobutrol in Gadobutrol solution for intravenous administration. Similarly chromatogram of placebo solution **Figure 3** showed no peaks at the retention time of Gadobutrol peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Gadobutrol in Gadobutrol solution for intravenous administration.

4.2 System suitability

System suitability was demonstrated by preparing standard solution as per the method and chromatographed the same into UPLC system in five replicated injections of standard solution. The System suitability was evaluated by computing the % Relative Standard Deviation for the peak area of these standard injections. System suitability data are summarized in **Table 1**.

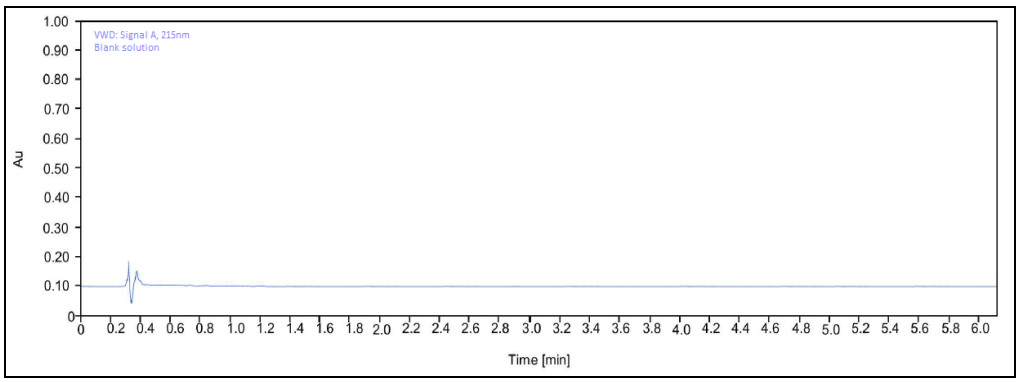


Figure 2. Chromatogram of blank

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

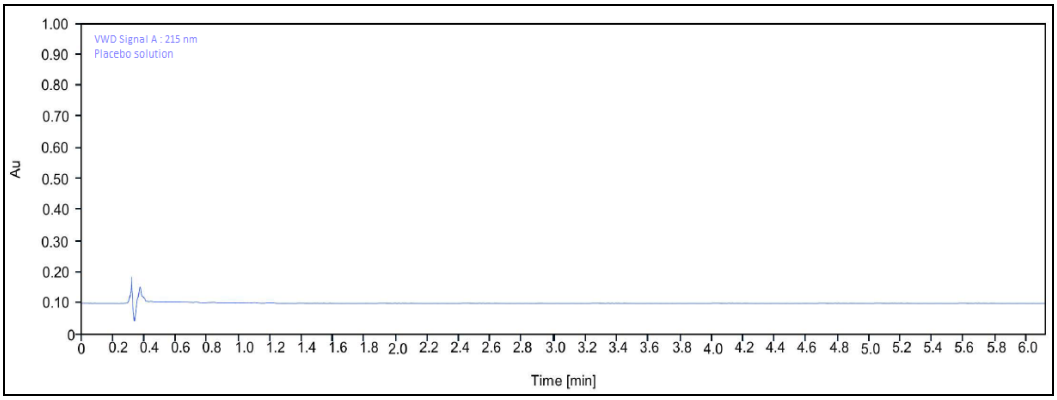


Figure 3. Chromatogram of placebo

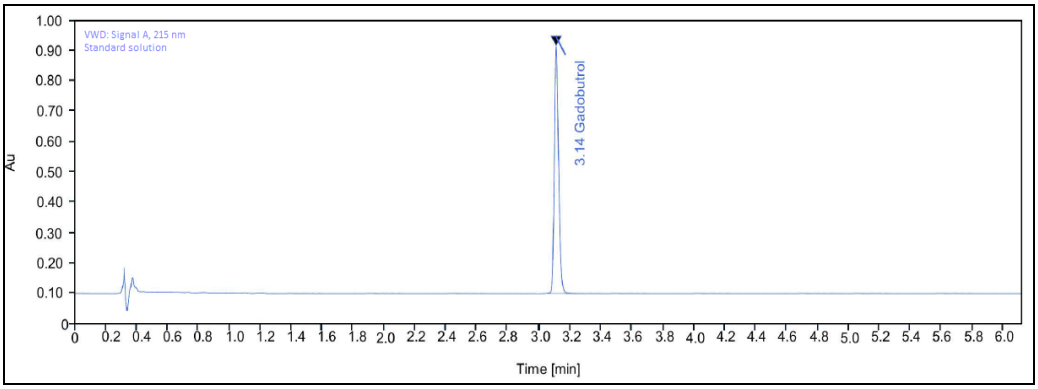


Figure 4. Chromatogram of Gadobutrol standard

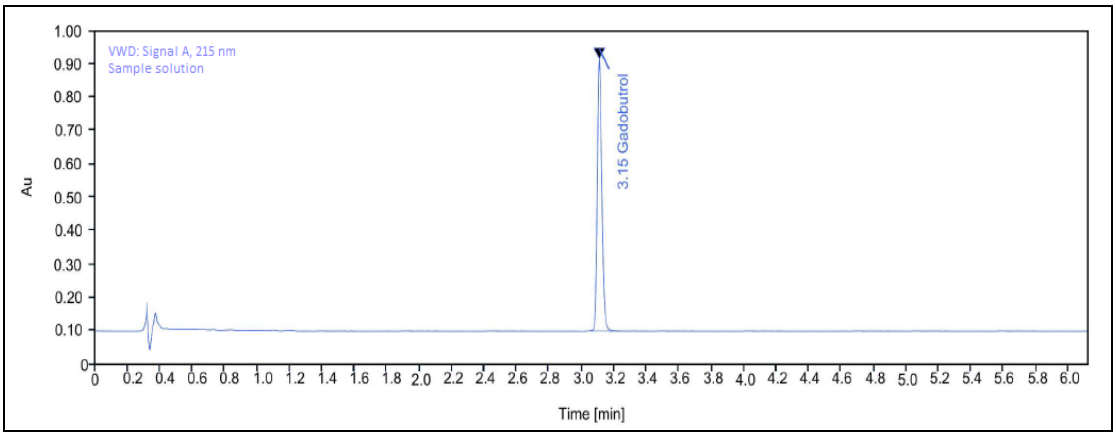


Figure 5. Chromatogram of Gadobutrol sample

Table 1. Specificity and System suitability results

Specificity				
S.No	Name	Retention Time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	3.14	No	No

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

4	Sample solution	3.15	No	No
System suitability				
S.No	Name	Retention time	USP Tailing	USP Plate Count
1	Gadobutrol	3.14	1.21	6155

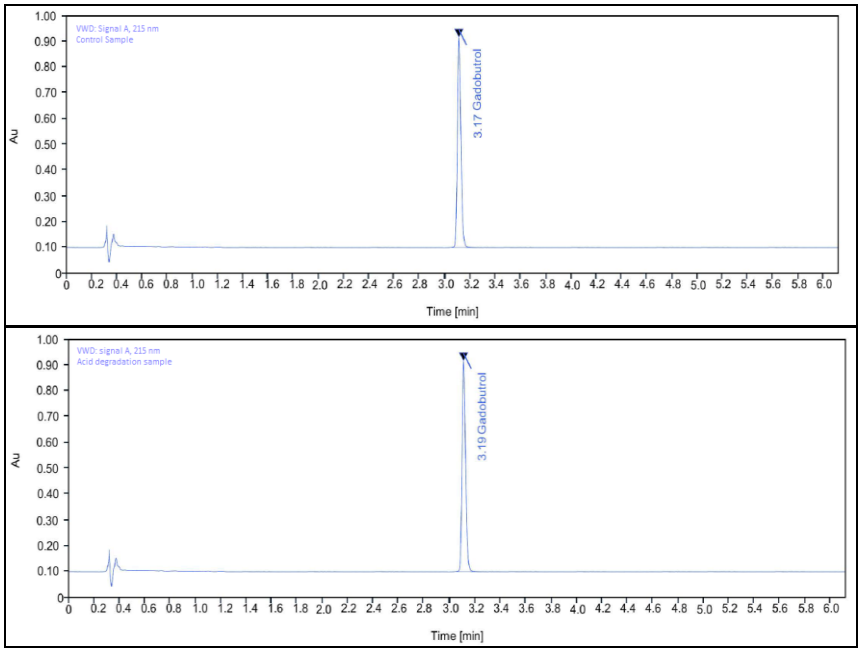
4.3 Force Degradation studies

A swot was carry out to demonstrate the successful parting of degradants/impurities as of Gadobutrol. Separate portions of sample and placebo solutions were exposed to the following stress conditions to induce degradation. Stressed and unstressed samples were injected into the UPLC system with a PDA detector. The degradation study results were presented in **Table 2**.

Table 2. Forced degradation results

Stress condition	Degradation condition	% Assay	% Degradation
As such	Control sample	100.2	NA
Acid	1.0 N HCl/60°C/48 Hrs	99.9	0.18
Alkali	1.0N NaOH/60°C/48 Hrs	99.5	0.21
Oxidative	30% H_2O_2/BT/48 Hrs	95.5	4.55
Photolytic	1.2 million Lux hours or 200 watt hours/m^2 for 7 days	99.8	0.22
Humidity	90%RH Exposed for 2 days	100.1	0.17
Thermal	105°C/2 days	100.3	0.19

Significant degradation was pragmatic in the (oxidative) peroxide stress conditions. Hence it can be finished that Gadobutrol is responsive to oxidation.



Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

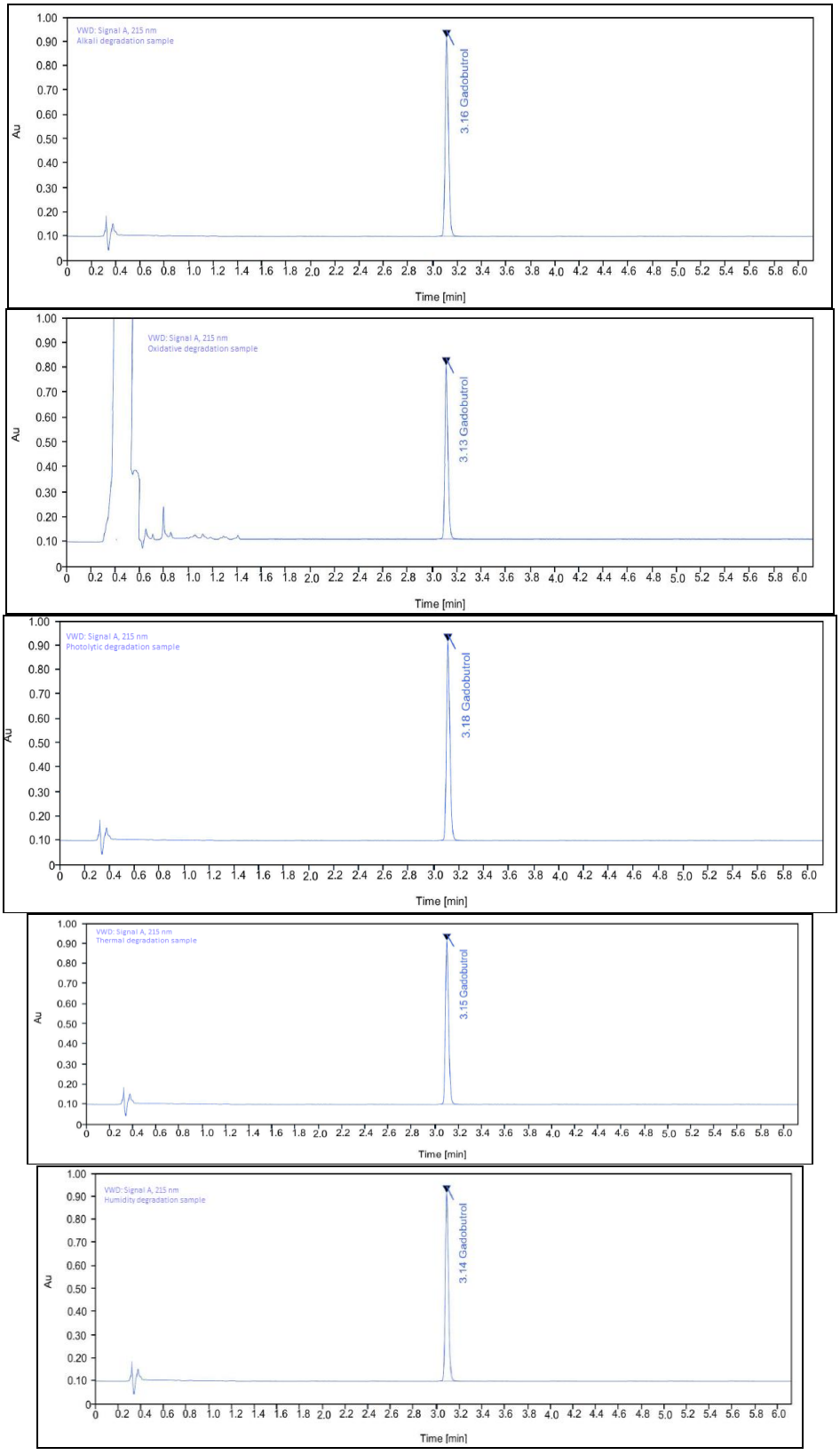


Figure 6. Stressed study chromatograms

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

4.4 System precision

The criterion solution was arranged as per the test technique, infused keen on the UPLC system six times, and calculated the % RSD for the vicinity responses. The statistics were revealed in **Table 3**.

4.5 Method precision

The precision of test method was evaluated by doing assay for six samples of Gadobutrol as per method. The content in mg and % label claim for Gadobutrol for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table 3.**

The relative standard deviation of six replicates criterion solution consequences were establish to be within the specification limit i.e.0.17%.

Table 3. System Precision and Method Precision results

System Precision			**Method Precision**	
S.No.	No.of injections	Peak area	No. of preparations	% Assay of Gadobutrol
1	Injection-1	7068949	Preparation-1	100.1
2	Injection-2	7039197	Preparation-2	99.8
3	Injection-3	7061861	Preparation-3	100
4	Injection-4	7054490	Preparation-4	99.9
5	Injection-5	7040555	Preparation-5	100.3
6	Injection-6	7049702	Preparation-6	99.5
Average		7052459	Average	99.93
STDEV		11744.186	STDV	0.2733
% RSD		0.17	%RSD	0.27

4.6 Linearity

The standard curve was obtained in the concentration range of 504.5-1513.5 µg/mL for Gadobutrol. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were calculated and given in **Figure 7** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table 4** the method was found to be linear within the proposed range.

Table 4. Linearity studies for Gadobutrol

S.No.	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 50%	504.50	3526051
2	Linearity at 75%	756.75	5209975
3	Linearity at 100%	1009.00	7052573
4	Linearity at 125%	1261.25	8915749

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

5	Linearity at 150%	1513.50	10588860
Correlation coefficient (r^2)			0.9996
Intercept			-73915.2000
Slope			7068.9364
% Y-intercept			-1.05

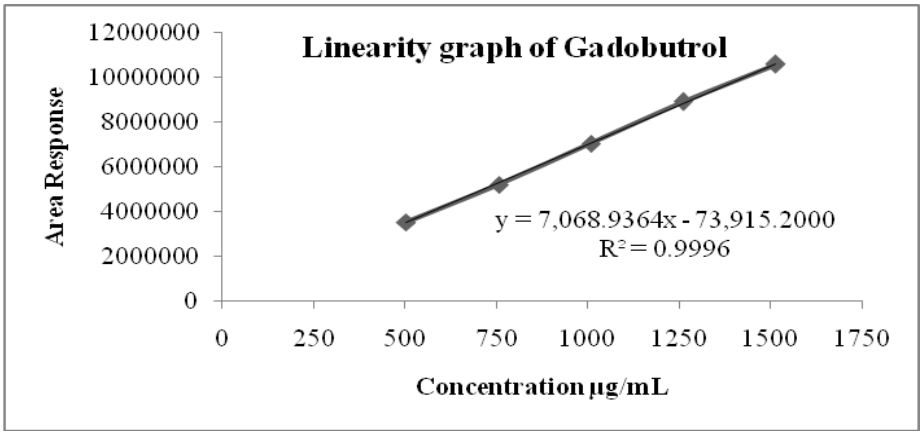


Figure 7. Calibration curve for Gadobutrol

4.7 Accuracy

The accuracy of the test technique was established by preparing revival samples of Gadobutrol at 50% to 150% of the target attentiveness echelon. The revival samples were organized in triplicate preparations on Gadobutrol API spiked to placebo, and analyzed as per the proposed method for each concentration level except 50% and 150 %. The statistics achieved which given in **Table 5**. and the technique was establish to be accurate.

Table 5. Recovery studies for Gadobutrol

% Level	(mg) Recovered	(mg) Added	% Recovery	Mean % Recovery
Accuracy at 50 %-1	50.42	50.59	99.7	99.6
Accuracy at 50 %-2	50.49	50.63	99.7	
Accuracy at 50 %-3	50.51	50.75	99.5	
Accuracy at 100 %-1	100.36	100.45	99.9	99.9
Accuracy at 100 %-2	100.49	100.64	99.9	
Accuracy at 100 %-3	100.41	100.55	99.9	
Accuracy at 150 %-1	150.57	150.32	100.2	100.1
Accuracy at 150 %-2	150.48	150.37	100.1	
Accuracy at 150 %-3	150.37	150.37	100.0	

4.8 Solution stability of analytical solutions

Solution constancy standards and sample solutions were established at an assortment of circumstances for instance bench top at room temperature and in refrigerator 2-8°C. The constancy of standard and sample solutions was establish by assessment of initially prepared criterion and sample solutions with

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 97-106 | ISSN:2251-6727

freshly prepared criterion solutions. The statistics were revealed in **Table 6 and Table 7.**

Table 6. Results for solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24 hrs	1.00	1.00
48 hrs	1.02	1.01

Table 7. Results for solution stability of sample solution

Sample at room temperature			sample in Refrigerator	
Time Interval	%Assay	%Assay difference	%Assay	%Assay difference
Initial	100.1	NA	100.1	NA
24 hrs	100.2	0.1	100.1	0.0
48 hrs	100.4	0.2	100.2	0.1

Standard and sample solutions are steady for 48 hrs when stored at room temperature and Refrigerator 2-8°C.

4.9 Robustness studies

Table 8. Robustness studies Results

Parameter		Theoretical plates	Tailing factor	%RSD of peak area
Flow variation ± 10%	0.45 mL	6951	1.2	0.14
	0.55 mL	6630	1.0	0.18
pH variation ± 0.2 units	3.6	5991	1.1	0.09
	4.0	6757	1.1	0.06
Temperature variation ± 5°C	35°C	6479	1.2	0.21
	45°C	6435	1.0	0.19
Organic phase variation ±10%	835:165	7182	1.2	0.11
	865.135	6108	1.0	0.31

The technique is robust for modify like flow rate, column oven temperature, and the organic phase of the mobile phase.

5. CONCLUSION

The developed method was validated to ensure the compliance in accordance with ICH guidelines. The method was found to be simple, selective, precise, accurate, and robust. The consequences obtained were within the acceptance criteria. So, it can be concluded

that the urbanized technique is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Gadobutrol in bulk and pharmaceutical dosage forms. Therefore, this method can be used for routine testing as well as stability analysis of Gadobutrol drug substance and drug products.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Chemistry, University college of sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Andhra Pradesh, India, for providing the facilities to carry this research work.

AUTHORS CONTRIBUTION STATEMENT

We have assured that "all authors have read and approved the manuscript." All the authors have equal contribution and participation in this research work. PRS and PB has analyzed all samples on UPLC instrument and completed the experimental work and was a major contributor in writing the manuscript. He had completed his work under the supervision of DRC a who help him to elaborate the methodology as well as theoretical approach.

FUNDING ACKNOWLEDGMENT

No funding was received.

CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

REFERENCES

[1] Cheng, K.T.; Gadobutrol. Molecular Imaging and Contrast Agent Database, 2007.

[2] Bayer in Radiology–Gadavist® (gadobutrol)injection1 mmol/mL. bayerimaging.com, 2015.

[3] FDA approves imaging agent for central nervous system scans. U.S. Food and Drug Administration (FDA), 2011.

[4] U.S. FDA Approves Bayer's Gadavist (Gadobutrol) Injection for MRI of the Central Nervous System. Bayer HealthCare Pharmaceuticals, 2011.

[5] Education for Cardiology Disease Professionals. touchcardiology.com. Radcliffe cardiology, 2015.

[6] Gadavist full prescribing information, 2011.

[7] Viv-Butrol-Vivere Imaging.

[8] Product information for Aus PAR Gadovist Gadobutrol Bayer Australia Ltd, 2015.

[9] Najana S. B.; Pokala R. K. V.; Bhavani K. G.; Khandapu B. M. K.; Bollikolla H. B. On the Stability Indicating Reverse Phase High-Performance Liquid Chromatography Method for Quantitative Estimation of Impurities in Gadobutrol Solution for Intravenous Administration. *Bioscience Biotechnology Research Communications*. 2022, *15(3)*, 435-441.

[10] ICH guidelines for validation of analytical procedures: text and methodology. Q2(R1) 2005.