Determination Of Trometamol Content In Gadobutrol Solution For Intravenous Administration, 1 mmol / mL Using RP-LC With Refractive Index Detector

Sanni Babu Najana (✉ n.sannibabu@gmail.com)  
Acharya Nagarjuna University

Hari Babu Bollikolla  
Acharya Nagarjuna University

Research Article

Keywords: Trometamol, Gadobutrol injection, RP-LC method, Refractive Index Detector, validation.

DOI: https://doi.org/10.21203/rs.3.rs-318563/v1

License: ☝️ This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

**Background:** Extremely responsive technique designed for the willpower of trometamol for instance tris(hydroxymethyl)aminomethane in gadobutrol by RP-LC technique. Quantification of trometamol content in gadobutrol samples by HPLC with refractive index detector (RID). Trometamol was UV inactive compound.

**Methods:** Trometamol was determined by RP-LC method using Inertsil NH₂ (250x4.6mm, 5µm) column as motionless segment. Column temperature maintained 30°C, inoculation quantity 10µL, flow velocity was 0.3 ml/min, sample cooler temperature 25°C. The mixture of phosphate buffer and cyanomethane in the ratio of 990:10 (volume/volume) was utilized as movable segment. The retention time of trometamol determined 10.95 minutes respectively. The acceptance limit of the trometamol content is 0.9%-1.5%.

**Conclusion**

The proposed RP-LC technique that can determination of trometamol content in gadobutrol solution intravenous administration contain expanded as well as authenticated as per ICH guidelines. The efficiency of the technique was ensure by the specificity, exactitude and accuracy. Hence, the technique well suit for their intended purposes and can be productively useful for the release testing of gadobutrol injection keen on the souk.

1. **Introduction**

Gadobutrol [1] 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) [2-5]. 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 C₁₈H₃₁GdN₄O₉. It has a relative molecular mass of 604.72 g/mol. Chemical structure shown in (Figure: 1.0).

Gadobutrol solution is a sterile, clear, colorless to pale yellow solution containing 604.72 mg gadobutrol per mL (equivalent to 1 mmol/mL) as the active ingredient and the excipients calcobutrol sodium, trometamol, hydrochloric acid (for pH adjustment) and water for injection. Gadavist contains no preservatives.

**Multifunctional characteristics of Tris amino in pharmaceutical products:**

- Buffering agent in both small and large volume parenterals
- Neutralization of carbomer polymers in topical gels or creams
Enhancement of antimicrobial action in contact lens solutions
Emulsifier when combined in situ with fatty acids
Formation of API salts to enhance solubility and bioavailability
Stabilization of API in tablet formulations by inhibiting acid-catalyzed degradation

Disadvantages of Tris amino:

Tromethamine pKa value 8.1 and typical buffer pH range 7.1 to 9.1.

Tromethamine (Figure: 2.0) was used in parenterals buffering agent. Discrepancy of the buffer pH, impacts mentioned below

- Decrease the solubility
- Bioavailability
- Antimicrobial action

1.1 Trometamol structure:

In literature, no analytical method was reported for the determination of Trometamol in Gadobutrol solution for intravenous administration. Hence the author was aimed towards the development of rapid, specific and robust methods for the determination of Trometamol in Gadobutrol solution for intravenous administration.

2. Experimental

Chemicals and reagents

Trometamol purchased from Merck India., Mumbai, India. Potassium dihydrogen ortho phosphate, milli-Q water and acetonitrile were procured from Merck, India.

Preparation of Buffer:

Dissolved 10.89g of KH$_2$PO$_4$ in 1000 mL of Milli-Q-water and sonicated to dissolve. Filter through 0.45µ membrane filter paper.

Preparation of Mobile phase: Buffer and Acetonitrile in the ratio of 990:10(%v/v).

Preparation of diluent:

Mobile phase used as diluent.

Preparation of Trometamol stock solution: Accurately weighed and transferred 60.0 mg of Trometamol standard into a 50mL volumetric flask, added 25 ml of diluent sonicated to dissolved. Then volume is made up to the mark with diluent.
**Standard solution:** Transferred 5 mL of Trometamol stock solution into a 50mL volumetric flask containing about 30mL of diluent. Mixed well and made up to the mark with diluent. This solution is equivalent to 120ppm of Trometamol with respect to 60.0mg/mL of sample solution.

**Preparation of sample spiked solution:**

Transferred 5 mL of Gadobutrol solution into a 50mL volumetric flask. Dissolved in 25mL of diluent and added 5mL of Trometamol standard stock solution. Mixed well and then made up to the mark with diluent.

**Chromatographic conditions**

RP-LC analysis was carried out on Waters 2690 separation module with Refractive index detector model:2414. Inertsil NH₂ (250x4.6mm, 5µm) column was used as stationary phase. The mixture of phosphate buffer and Acetonitrile in the ratio of 990:10 (v/v) was used as mobile phase. The flow rate of the mobile phase was kept at 0.3 mL/min. The injection volume was set as 10µL. Column oven temperature and auto sampler temperature were set as 30°C and 25°C, respectively. Refractive index detector temperature 30°C and sensitivity 1028.

**3. Results & Discussion**

3.1. **Method development**

A Spiked solution containing Trometamol and Gadobutrol solution was run in 0.5 mL/min flow rate. Trometamol peak closely eluted to placebo peak and hence the flow rate of the mobile phase was decreased from 0.5 mL/min to 0.3 mL/ min. In this condition Trometamol peak eluted at an optimum retention time, placebo peak and Trometamol peaks are well separated. Hence, the elution order was observed from the chromatogram *(Figures.3.0 to 7.0).*

3.2. **Method validation** [6]

3.2.1 **Specificity**

**Blank interference:**

Blank was prepared and injected as per test method. It was observed that no blank peaks were interfering with analytical peaks. Figure shown in **Figure: 8.0**.

**Placebo interference:**

Placebo solutions were prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering with analytical peaks. Figure shown in **Figure: 9.0**.
It was observed that no interference of blank and placebo at trometamol retention time. Figure shown in Figure: 10.0.

3.3 Precision

3.3.1 System Precision:

Perform the analysis of Diluted standard six times and determine the percentage relative standard deviation of peak area, Asymmetry and Theoretical plates of replicate injections of Trometamol. System precision results is tabulated in Table: 1.0.

| S.No. | Peak area | Asymmetry | Theoretical plates |
|-------|-----------|-----------|--------------------|
| 1.    | 43104     | 1.12      | 18443              |
| 2.    | 43309     | 1.13      | 18401              |
| 3.    | 43456     | 1.13      | 18354              |
| 4.    | 43066     | 1.14      | 18500              |
| 5.    | 43286     | 1.13      | 18499              |
| 6     | 43100     | 1.13      | 18528              |
| Average | 43220   | NA        | NA                 |
| STDEV | 154.6252 | NA        | NA                 |
| % RSD | 0.40      | NA        | NA                 |

The % relative standard deviation for peak area of Trometamol peak from six replicate injections of standard solution 0.4%. The asymmetry for Trometamol peak from standard solution is 1.12

The theoretical plates for the Trometamol peak from standard solution is 18443.

3.3.2 Method Precision

Precision was determined by injecting six sample solutions spiked Trometamol at specification level. The samples were prepared as per the method and the result for precision study is tabulated in Table: 2.0.

| S.No. | Peak area | Asymmetry | Theoretical plates |
|-------|-----------|-----------|--------------------|
| Average | 43220   | NA        | NA                 |
| STDEV | 154.6252 | NA        | NA                 |
| % RSD | 0.40      | NA        | NA                 |
The method precession was performed with six replicate solutions prepared and the results found within the acceptance criteria.

### 3.4 Linearity and Range

The linearity is determined by injecting the solutions in duplicate Trometamol ranging from 25% to 150% of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Linearity results is tabulated in Table: 3.0. Figure shown in Figure: 11.0.

**Table: 3.0 Linearity of detector response 2,3-Dimethylaniline**

| Level | Concentration (PPM) | Mean Area |
|-------|---------------------|-----------|
| 25    | 29.8251             | 10499     |
| 50    | 59.6503             | 20954     |
| 75    | 89.4754             | 31981     |
| 100   | 119.3005            | 42233     |
| 125   | 149.1257            | 52839     |
| 150   | 178.9508            | 64342     |

- Correlation coefficient: 0.9999
- % Y-intercept: -0.9
- Slope: 359.3536
- Intercept: -370.8618

### 3.5 Accuracy

Recovery of Trometamol was performed. The sample was taken and varying amounts of Trometamol representing 50% to 150 % of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in Table 4.0.

**Table: 4.0 Accuracy study of Trometamol**

| Inj. No | Trometamol content |
|---------|--------------------|
| 1       | 1.15               |
| 2       | 1.15               |
| 3       | 1.15               |
| 4       | 1.16               |
| 5       | 1.15               |
| 6       | 1.15               |

| Mean (%) | 1.15 |
|----------|------|
| SD       | 0.0041 |
| % RSD    | 0.4 |
### % Recovery

| % Level         | % Recovery | Mean % Recovery | STDEV  | % RSD |
|-----------------|------------|----------------|--------|-------|
| 50% sample-1    | 93.4       | 93.7           | 2.1197 | 2.3   |
| 50% sample-2    | 91.8       |                |        |       |
| 50% sample-3    | 96.0       |                |        |       |
| 100% sample-1   | 97.6       | 100.0          | 2.3065 | 2.3   |
| 100% sample-2   | 100.2      |                |        |       |
| 100% sample-3   | 102.2      |                |        |       |
| 150% sample-1   | 103.2      | 103.7          | 0.6429 | 0.6   |
| 150% sample-2   | 104.4      |                |        |       |
| 150% sample-3   | 103.4      |                |        |       |

#### 3.6 Solution stability of analytical solutions:

Trometamol standard and sample solutions were kept for about 48 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of “old” prepared standard solutions with freshly prepared standard solutions. Solution stability results is tabulated in **Table: 5.0 to 8.0.**

**Table: 5.0 Results for solution stability of standard at room temperature**

| Time Interval | % Recovery for standard solution |
|---------------|---------------------------------|
| Initial       | -                               |
| 24hrs         | 100.5                           |
| 48hrs         | 101.1                           |

**Table: 6.0 Results for solution stability of standard in Refrigerator**

| Time Interval | % Recovery for standard solution |
|---------------|---------------------------------|
| Initial       | -                               |
| 24hrs         | 101.1                           |
| 48hrs         | 101.3                           |

**Table: 7.0 Results for solution stability of standard at room temperature**

| Time Interval | % Recovery for sample solution |
|---------------|-------------------------------|
|               | % Assay                       |
| Initial       | NA                            |
| 24hrs         | 100.0                         |
| 48hrs         | 100.9                         |
Table: 8.0 Results for solution stability of standard in Refrigerator

| Time Interval | % Recovery for sample solution |
|---------------|--------------------------------|
|               | % Assay                        |
| Initial       | NA                             |
| 24hrs         | 100.0                          |
| 48hrs         | 100.0                          |

The standard and sample solutions are stable up to 48 Hrs on bench top and when stored in refrigerator (2-8°C) condition.

3.7 Robustness

To validate the method robustness the chromatographic performance at changed conditions was evaluated compared to nominal conditions of the method. Standard solution was injected at each of the following changed conditions: Robustness results is tabulated in Table:9.0.

Table: 9.0 Results of Robustness

| Parameter                        | Altered condition | Retention time (minutes) | Asymmetry | Theoretical plates | %RSD | Trometamol content (mg/ml) |
|----------------------------------|-------------------|--------------------------|-----------|--------------------|------|----------------------------|
| Mobile phase variation in ratio  | (0.9:99.1)        | 12.610                   | 1.07      | 16446              | 0.5  | 1.13                       |
| Mobile phase variation in ratio  | (1.1:98.9%)       | 12.593                   | 1.06      | 16511              | 0.3  | 1.14                       |
| Flow variation (0.3 mL/min)      | (0.4 mL/min)      | 9.300                    | 1.09      | 15563              | 0.3  | 1.16                       |
| Temperature variation (30°C)     | 25°C              | 12.424                   | 1.11      | 16939              | 0.4  | 1.15                       |
| Temperature variation (30°C)     | 35°C              | 12.297                   | 1.11      | 16684              | 0.6  | 1.15                       |

The developed method was applied for the determination of Trometamol content in Gadobutrol in injection formulations obtainable in the local market. The test injection sample was prepared at a concentration of 60.0mg/mL and analyzed by the proposed method three times. The percent recovery and %RSD of Trometamol was calculated. The mean percent recovery and relative standard deviation of Gadobutrol was found to be 95.24% and 0.50% (Table 10).

Table: 10.0 Trometamol content of Gadobutrol in injection formulation.

| Trometamol content (Label claim Gadovist 604.72 mg/mL, 1 mmol/mL) | Trometamol content | Recovery | Mean recovery | SD | %RSD |
|------------------------------------------------------------------|-------------------|----------|---------------|----|------|
| 1.211                                                            | 1.15              | 94.96    | 95.24         | 0.47675 | 0.50  |
| 1.211                                                            | 1.16              | 95.79    |               |    |      |
| 1.211                                                            | 1.15              | 94.96    |               |    |      |
4. Conclusions

The proposed RP-LC method that can determination of trometamol content in Gadobutrol solution intravenous administration have been developed and validated as per ICH guidelines. The effectiveness of the method was ensure by the specificity, precision and accuracy. Hence, the method well suit for their intended purposes and can be successfully applied for the release testing of Gadobutrol injection into the market.

Abbreviations

RP-HPLC: Reverse phase high performance liquid chromatography, ICH: International Conference on Harmonization, RSD: Relative standard deviation, RID: refractive index detector.

Declarations

Ethics approval and consent to participate (Human, Animals, Plants and Source):

In this article, the authors have performed no experiments with any of the animal and human subjects.

Consent for publication:

Not applicable

Availability of data and materials:

All data and material are available upon request.

Competing interests:

Not applicable

Funding:

No funding was received

Authors' contributions:

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

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

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Product information for Aus PAR Gadovist Gadobutrol Bayer Australia Ltd PM-2013-04968-1-2 Final 1 June 2015.
2. Cheng KT (2007) "Gadobutrol" (PDF). Molecular Imaging and Contrast Agent Database (MICAD). National Center for Biotechnology Information (NCBI). (2007).
3. "Bayer in Radiology–Gadavist® (gadobutrol) injection 1 mol/mL". bayerimaging.com. Retrieved 20 May 2015.
4. "FDA approves imaging agent for central nervous system scans"(Press release). S. Food and Drug Administration (FDA). March 15, 2011. Retrieved March 31, 2011.
5. "U.S. FDA Approves Bayer's Gadavist (Gadobutrol) Injection for MRI of the Central Nervous System" (Press release). Bayer HealthCare Pharmaceuticals. March 14, 2011. Archived from the original on May 2, 2011. Retrieved March 31, 2011.
6. International Conference on Harmonisation guidelines (2005) on validation of analytical procedures, Q2 (R1).

Figures

![Chemical structure of Gadobutrol](image)

Figure 1

Chemical structure of Gadobutrol
Figure 2

Chemical structure of Trometamol (tris(hydroxymethyl)aminomethane)

Figure 3

typical chromatogram of Blank

Figure 4
typical chromatogram of Placebo

Figure 5

typical chromatogram of Standard

Figure 6

typical chromatogram of Test sample
Figure 7

Spiked Trometamol chromatogram of Gadobutrol

Figure 8

typical chromatogram of Blank
Figure 9

typical chromatogram of Placebo

Figure 10

Spiked Trometamol chromatogram of Gadobutrol
Figure 11

Linearity of detector response for Trometamol