Research Article

A Novel Potentiometric Titration Method for Quantitative Determination of Bromide Content in Doxorubicin Hydrochloride

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ABSTRACT

A simple precise, rapid accurate and sensitive Potentiometric titration method was developed for quantitative determination of Bromide content in Doxorubicin Hydrochloride. The titration was carried out by using standardized 0.1 N Silver Nitrate solutions ($AgNO_3$). To confirm the presence of bromide content spiking study was carried out by spiking 0.1% NaBr into the test preparations which shows the 0.1 % increase in the % Cl of the actual % Cl. The end point was determined by using Mettler T 50 Auto titrator with DM 141 SC electrode. The proposed method was found to be precise with % RSD <1 (n = 6). The method showed strict linearity ($r^2 > 0.999$) between 0.5% to 4.0%. The percentage recovery of Bromide in the optimized method was between 96.3 % to 96.7 %.

Keywords: Bromide content, doxorubicin hydrochloride, hydrobromic acid (HBr), potentiometric titration, silver nitrate ($AgNO_3$),

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INTRODUCTION

Doxorubicin is a drug used in cancer chemotherapy. It is an anthracycline antibiotic, closely related to the natural product daunomycin, and like all anthracyclines, it works by intercalating DNA, with the most serious adverse effect being life-threatening heart damage. It is commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas [1]. The drug is administered intravenously, as the hydrochloride salt. Doxorubicin is commonly used to treat some leukemias and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others [2]. Doxorubicin interacts with DNA by intercalation and inhibition of macromolecular biosynthesis [3, 4]. This inhibits the progression of the enzyme topoisomerase II, which relaxes supercoils in DNA for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication. The planar aromatic chromophore portion of the molecule intercalates between two base pairs of the DNA, while the six-membered daunosamine sugar sits in the minor groove and interacts with flanking base pairs immediately adjacent to the intercalation site, as evidenced by several crystal structures [5]. The manufacturing process of Doxorubicin involves use of Bromine or Hydrobromic acid and the final drug applied as its Hydrochloride salt (**Fig. 1**). Due to poisonous effect of Bromine its presence cannot be neglected. So during our research work we established a novel and precise Titrimetric procedure for quantitative determination of Bromide content in Doxorubicin Hydrochloride.

MATERIALS AND METHODS

Instrumentation

Potentiometric Titrator: Mettler T-32
Analytical Micro balance: Mettler XP 26

Reagents and chemicals

Reagents and chemicals used are mentioned in (**Table 1**)

Table 1: Reagents and Chemicals

Sr.No.	Reagents and Chemicals	B. No./Manufacturer	Purity/Assay
01.	$AgNO_3$	3255763 Spectrochem	99.9 %
02.	Doxorubicin HCl	In House	99.52 %
03.	NaCl	I06F/0706/2007/46 S D Fine	99.9 %
04.	NaBr	I08Z/0708/2007/21 S D Fine	99.0 %

General procedure

Standardization of 0.1 N $AgNO_3$

About 100 mg of NaCl (previously powdered lightly, dried at 120°C for 2 hours) was weighed accurately into clean and dry titration cup. It was dissolved in 25 ml of water It was titrated with 0.1 N $AgNO_3$ solution determining the end point potentiomatrically using DM 141 SC electode. Blank determination was performed out for necessary correction. The titration was performed in duplicate.

One ml of 0.1 N $AgNO_3$ is equivalent to 0.585 gm of sodium chloride (NaCl)

Normality of $AgNO_3$ = $\frac{W}{B.R \times 0.585}$

Where,
W is weight of NaCl in g.
B.R. is burette reading in ml.

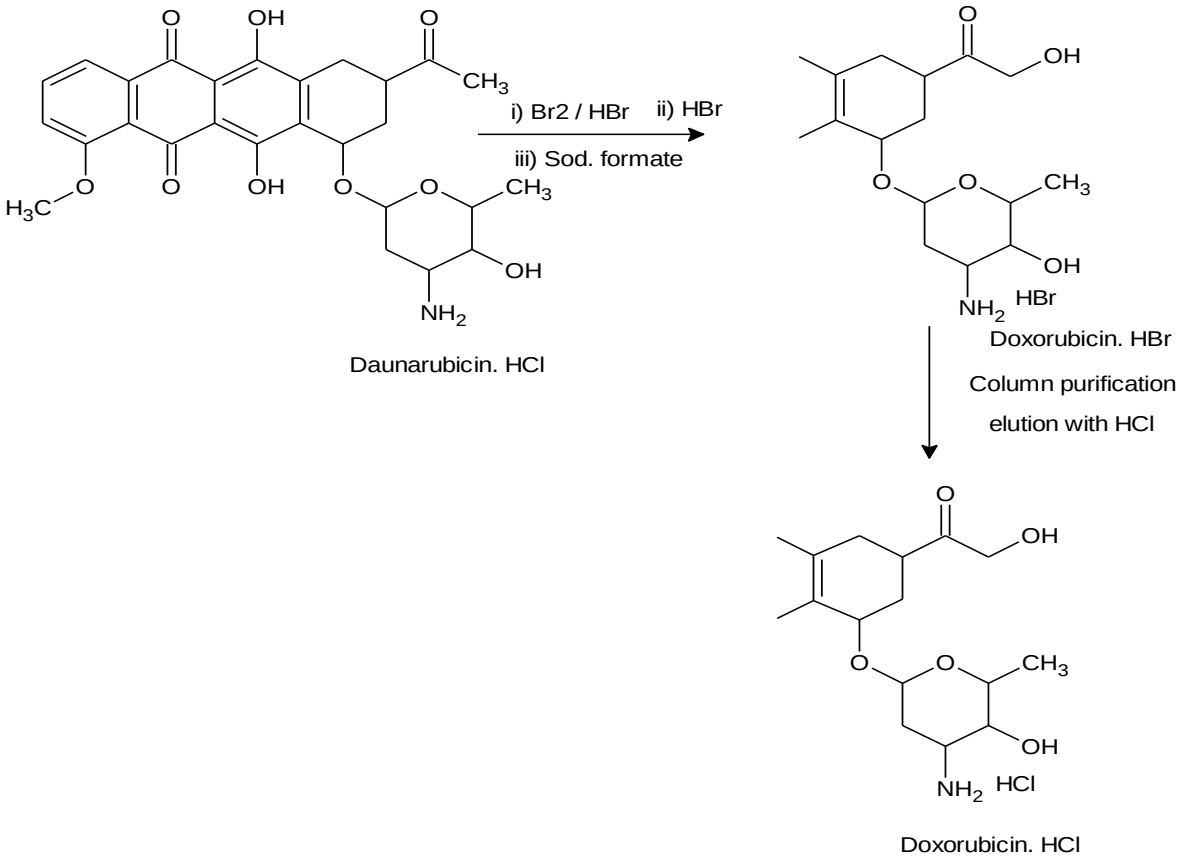

Figure 1: Doxorubicin HCl reaction Scheme

Quantitative determination of HBr Content

About 0.1 g. of Doxorubicin HCl test sample was weighted accurately into a clean and dried titration cup. It was dissolved in 25 ml. of water. It was titrated against 0.1N $AgNO_3$ solution using DM 141 SC Electrode by Auto Titrator. Blank determination was also carried out for necessary correction.

One ml of 0.1 N $AgNO_3$ is equivalent to 79.904 g. of HBr

$$\% \text{ HBr Content} = \frac{(T_1 - B_1) \times N \times F \times 100 \times 10}{W \text{ (in gm)} \times 25 \times 1000}$$

Where,

B_1 – Burette reading of Blank titration

T_1– Burette reading of Test titration

N –Normality of 0.1N $AgNO_3$ volumetric solution

F – Factor of HBr (79.904 gm/mole)

W – Weight of Test Sample in gm

RESULTS AND DISCUSSION

During the synthesis of Doxorubicin HCl from Daunorubicin; HBr is used in the earlier steps of the reaction. It is converted into HCl by column purification. And the final product is expected to be a hydrochloride salt of Doxorubicin but sometimes some part of HBr is not completely converted in to HCl that effects to the actual content of the resultant Doxorubicin percentage. So the proposed method is developed for quantitative determination of remaining HBr content in doxorubicin hydrochloride.

Determination of Bromide content

The objective of this work was to determine accurately the content of Bromide. The content of Bromide for three different preparations was analyzed using the above method. It was in the range of 96.3 % to 96.7 %.

Analytical method validation

Method Precision

The method precision was checked after analyzing six different preparations of homogeneous test sample of Doxorubicin. The study shows that bromide is not detected. The % RSD of results obtained was found 0.93. It confirms good precision of the method (**Table 2**). To confirm the presence of bromide content spiking study was carried out by spiking 0.1% NaBr (**Fig.2, 3 & 4**) into the test preparations which shows the 0.1 % increase in the % Cl of the actual % Cl. This conform the presence of bromide in the test preparation (**Table 3**).

Table 2: Method Precision

Sr. No.	Weight of sample in gm	B. R of $AgNO_3$ in ml for Br content	B. R of $AgNO_3$ in ml for Cl content	%(w/w) Bromide content	% (w/w) Cl content
1	0.10124	ND	3.41	ND	5.97
2	0.10105	ND	3.33	ND	5.84
3	0.10675	ND	3.59	ND	5.96
4	0.10120	ND	3.42	ND	5.99
5	0.10175	ND	3.42	ND	5.96
6	0.10130	ND	3.42	ND	5.98
Average			3.43		5.95
Std.dev			0.085		0.055
% RSD			2.48		0.93

Table 3: Method Precision (Spike study)

Titration sets	Weight of Doxorubicin HCl (g)	Volume of $AgNO_3$ consumed (ml)	% Cl Content
Sample 1	0.10227	3.4849	6.04
SPL 1 + 0.1 % NaBr	0.10363	3.7685	6.45
Sample 2	0.10104	3.4333	6.02
SPL 2 + 0.1 % NaBr	0.10880	3.9537	6.44
Sample 3	0.10352	3.5306	6.05
SPL 3 + 0.1 % NaBr	0.10145	3.6763	6.42

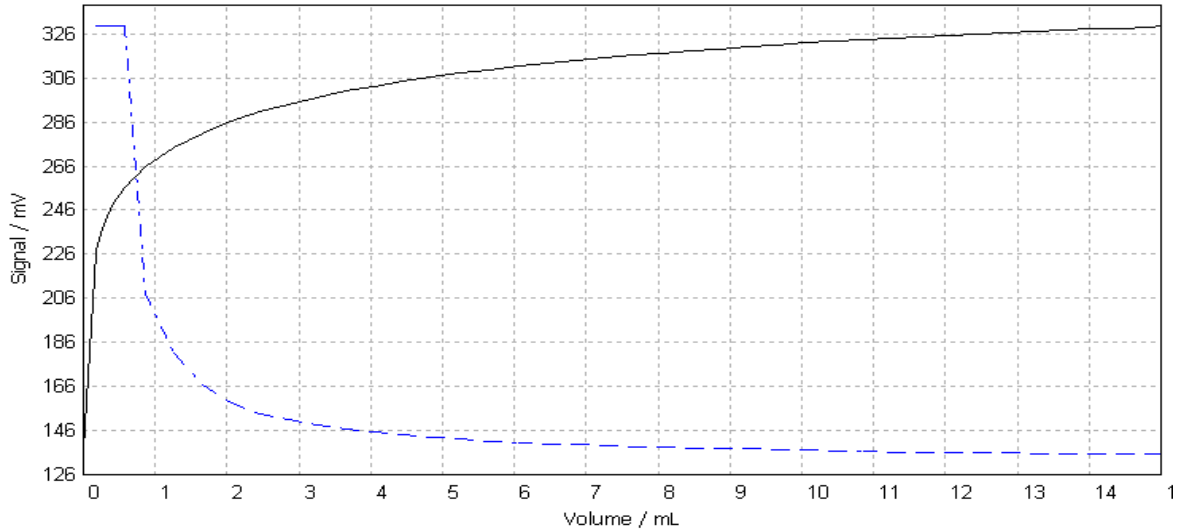

Figure 2: Blank

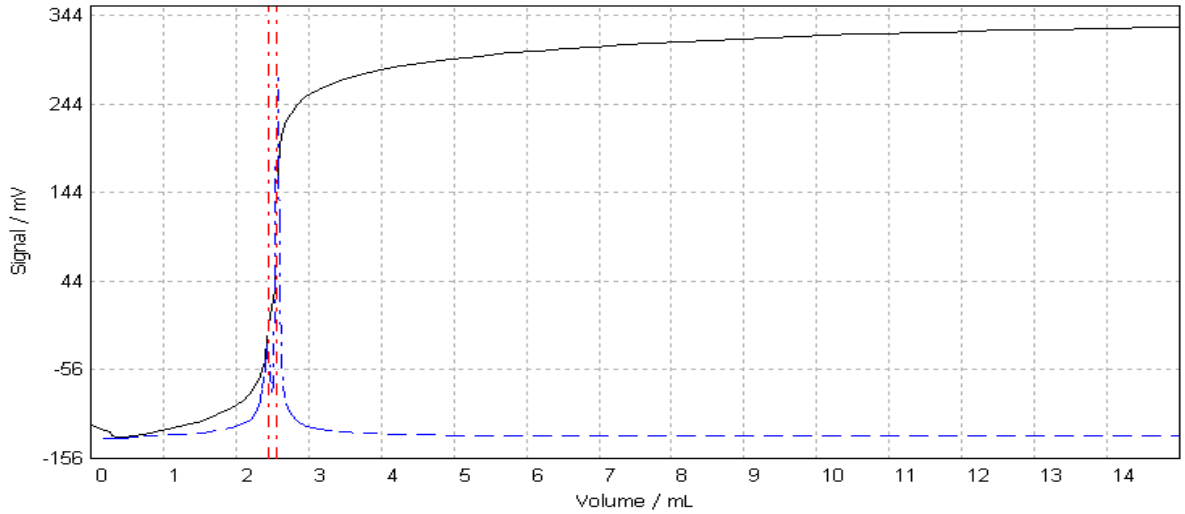

Figure 3: Sample

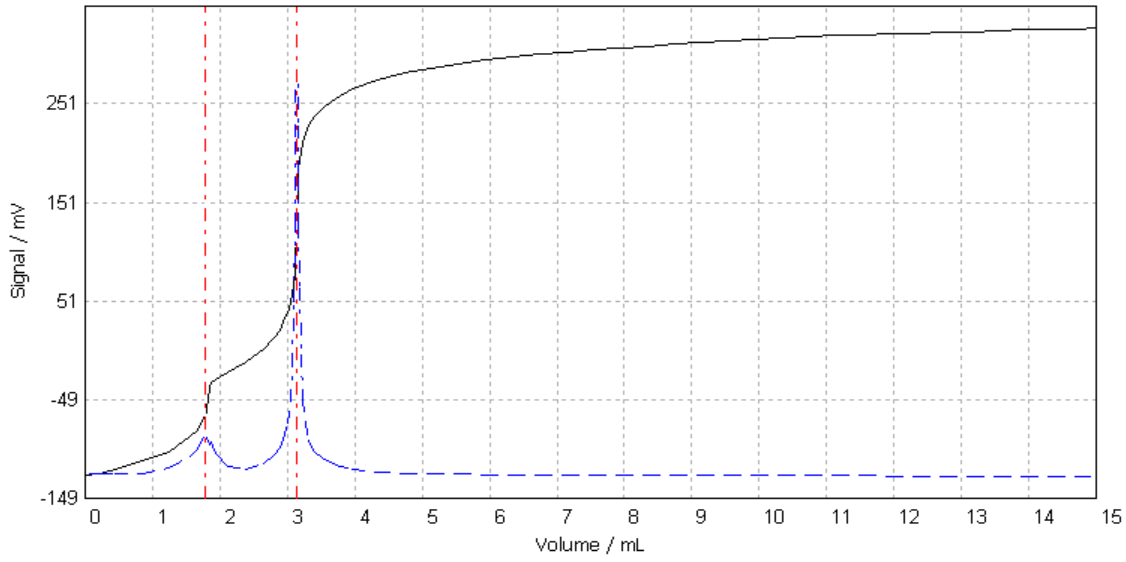

Figure 4: NaBr spiked with Sample

Linearity

For the establishment of method linearity five different weights of Doxorubicin HCl test samples corresponding to 0.5%, 1.0%, 2.0%, 3.0% and 4.0 % of the weight (0.1 g) were taken and analyzed for % of bromide content (**Table 4**).

Table 4: Linearity

Titration sets	Concentration (%)	Ml of $AgNO_3$	Bromide (% w/w)
1	0.500	1.965	59.70
2	1.010	4.068	61.18
3	2.007	8.011	60.65
4	3.017	12.231	61.61
5	4.003	16.520	62.70

The Potentiometric titration was conducted at each level. Linearity curve was drawn (**Fig.5**) by plotting sample concentration on X axis and values of ml of $AgNO_3$ consumed on Y axis. And the regression values are calculated (**Table 5**).

Table 5: Regression values

Parameters	Bromide
Intercept	-0.16
Slope (S)	4.14
Correlation	0.9998
Co efficient Correlation R^2	0.9997

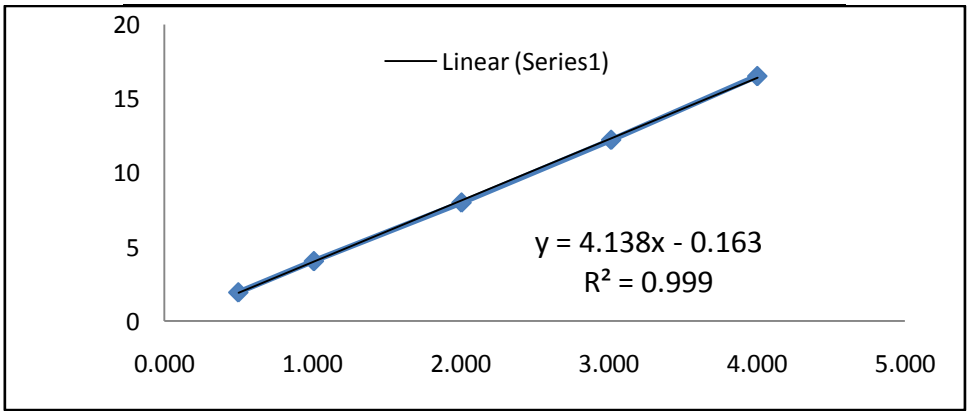

Fig.5: Linearity Curve

Accuracy and recovery

Accuracy was determined at three different levels i.e., 80 %, 100 % and 120 % of the nominal concentration. (0.1 g) The titration was conducted in triplicate at each level and the titer value was recorded. The tire value obtained in linearity study was considered as true value during the calculation of percentage (%) recovery. The percentage range recovery of Bromide was found in the range of 96.3 % to 96.7 %. It confirms the accuracy of the proposed method (**Table 6**).

The percentage recovery is calculated using following equation.

$$\% \text{ Recovery} = \frac{\text{Bromide content in test solution after spike X 100}}{\text{Actual Bromide content in as such solution prepared}}$$

Table 6: Accuracy/Recovery

% Level	Concentration. (%)	Ml of $AgNO_3$	Bromide (% w/w) After spike	Recovery (%)
80 %	75.431	18.18	72.63	96.3
100 %	76.044	22.72	72.78	95.7
120 %	75.056	27.25	72.60	96.7

LOD-LOQ Determination

LOD-LOQ was determined at five different levels i.e., 0.1 % to 0.005 % of the nominal concentration (0.1 g). The titration was conducted at each level and the titer value was recorded. The limit of detection obtained is 0.00135 % and the limit of quantization obtained is 0.00410 % (**Table 7**).

Table 7: LOD-LOQ Determination

Parameters	Bromide
Standard Deviation(σ)	0.01
Slope (S)	24.37
Limit of detection (%)	0.00135
Limit of Quantitation (%)	0.00410

CONCLUSION

The proposed method of Potentiometric titration was found to be precise, accurate and linear. The values of percentage recovery and standard deviation showed sensitivity. The method was validated. It showed satisfactory data for all the parameters of validation. Hence it can be applied for routine quality control application.

REFERENCES

1. Laginha, K.M. "Determination of Doxorubicin Levels in Whole Tumor and Tumor Nuclei in Murine Breast Cancer Tumors." Clinical Cancer Research. October 1, 2005. Vol. 11 (19). Retrieved on April 19, 2007.
2. Doxorubicin (Systemic)." Mayo Clinic. Last updated on: June 15, 1999. Retrieved on April 19, 2007. Archived April 3, 2007 at the Wayback Machine
3. Frederick CA, Williams LD, Ughetto G, et al. (March 1990). "Structural comparison of anticancer drug-DNA complexes: adriamycin and daunomycin". Biochemistry 29 (10): 2538–49.
4. Momparler RL, Karon M, Siegel SE, Avila F (August 1976). "Effect of adriamycin on DNA, RNA, and protein synthesis in cell-free systems and intact cells". Cancer Res 36 (8): 2891–5
5. Pigram WJ, Fuller W, Hamilton LD (January 1972). "Stereochemistry of intercalation: interaction of daunomycin with DNA". Nature New Biol 235 (53): 17–9.