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ESTIMATION OF UNCERTAINTY FOR MEASURING GALANTAMINE HYDROBROMIDE IN PHARMACEUTICAL FORMULATION USING ULTRAVIOLET SPECTROPHOTOMETRY

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ABSTRACT

Analytical results play a vital role in quality control of bulk drugs and pharmaceutical formulations. The main aim of this proposed research work is to study uncertainty estimation for quantitative determination of Galantamine hydrobromide from pharmaceutical formulation using ultraviolet spectrophotometry. The estimation of uncertainty was performed using cause effect (Ishikawa) diagram. Determination of uncertainty components proved to be a good way for experimental model to obtain low contribution of uncertainty to analytical results. This research paper explains identification of uncertainty sources, starting from a clear declaration of measured species, quantification of these uncertainty sources and a combination of these individual sources to estimate standard and expanded uncertainty. Determination of various components of uncertainty is a best method to confirm that results obtained of analytical methods are certain. It is concluded from the present study that uncertainty estimation for assay of Galantamine hydrobromide from pharmaceutical formulation is influenced by sample concentration rather than volumetric flask and sample mass. Thus sample concentration is the major factor to achieve precise results of the analysis.

Keywords: *Galantamine hydrobromide (GH), Uncertainty estimation, Cause effect (Ishikawa) diagram, Ultraviolet spectrophotometry.*

INTRODUCTION

Galantamine hydrobromide (GH, Fig. 1) is widely used for the treatment of mild to moderate Alzheimer's disease and various memory impairments [1-4]. Its molecular weight is 368.27, melting point is 258-264 °C and it is very soluble in water [5]. USP describes HPLC assay method for determination of GH [6]. There are several other methods reported for estimation of GH in bulk drug and pharmaceutical formulation like Zero order derivative[7] and first order derivative UV spectrophotometry[7,8] and tandem mass spectrometry[9].

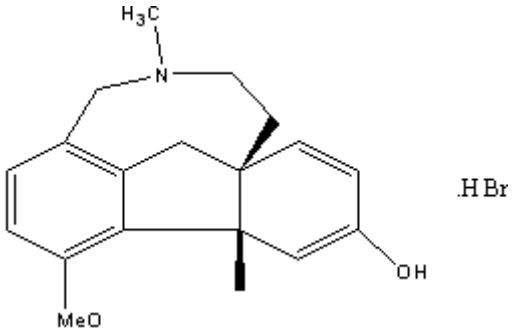

Fig. 1: *Structure of galantamine*

Reliable analytical methods are needed in all the fields of chemical analysis in order to achieve precise results. A measurement is complete only if it includes uncertainty estimation test. There are some reports which described the estimation of analytical uncertainty measurement [10-13]. These reports have explained identification of uncertainty sources, starting from a clear declaration of measured species, quantification of these uncertainty sources and a combination of these individual sources to estimate standard and expanded uncertainty. The present study describes results of uncertainty for estimation of GH in pharmaceutical formulation using ultraviolet spectrophotometry based on cause-effect Ishikawa[10] type diagram.

EXPERIMENTAL

Instruments and reagents:

Spectrophotometric measurements were made on a Shimadzu 1700 double beam UV-VIS spectrophotometer with a fix slit width of 1 nm coupled with Shimadzu UV PC software (UV probe) version 2.31. Double distilled water was used throughout the study and pure GH was obtained from Alembic Pharmaceuticals Ltd. Vadodara, India. The marketed

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formulation of GH was obtained commercially from Sun Pharmaceuticals Ltd (Galmer®-40 tablets labelled as 4 mg of pure drug, Batch Number-GK 90964).

Procedure

The calibration curve for GH determination was obtained by using six standard solutions in the range of 8.1×10^{-5} - 2.1×10^{-4} mol/l in double distilled water. Sample solution was prepared in same manner using marketed formulation of GH. The absorbance was measured at $ë_{max}$ = 287 nm. Uncertainty evaluation requires (a) specification of measured analytes; (b) a clear and ambiguity free declaration of what is measured and (c) a quantitative expression that links the value of measured analytes to the parameters on which it depends. In order to list the uncertainty sources, it is very convenient to use cause-effect (Ishikawa) diagram because it shows how sources link to each other, indicating their influence on the results. The cause-effect diagram describes main uncertainty sources of the process. Main branches represent parameters those influence the results. These parameters are presented in Eq. (1):

$$Q_{sample} = C_{10}\, V_{10}\, 10^{-6}/m_{sample} \qquad (1)$$

Where, Q_{sample} – quantity of analyte (mol/kg); C_{10} – concentration of analyte in the 10 ml volumetric flask (mol/l); V_{10} – volume of the 10 ml volumetric flask (ml); and m_{sample} – sample (GH) mass taken (kg).

After identifying various uncertainty sources, next step is to quantify uncertainty and last step is to calculate an expanded uncertainty, choosing a coverage factor.

Different uncertainty sources

Uncertainty due to concentration, C_{10}

Concentration of GH was determined using an established calibration curve. The stock solution was prepared by weighing 10 mg of GH in 10 ml of volumetric flask and diluting it to 10 ml with double distilled water. Six standard solutions were prepared by sub diluting stock solution with double distilled water to get the concentration range of 8.1×10^{-5}-2.1×10^{-4} mol/l. All the six standard solutions were measured three times. In this case, the uncertainty due to standard solution was low enough to be neglected. The uncertainty of sample solution (unknown sample) is given by Eq. (2):

$$\mathbf{u(c)} = \frac{Sr}{b}\sqrt{\frac{1}{n}+\frac{1}{p}+\frac{(c-m)^2}{Sxx}} \qquad (2)$$

$$\text{Where } Sr = \sqrt{\frac{\sum_{j=1}^{n}[Yj-(bxi+a)]^2}{n-2}}$$

Sr – Residual standard deviation; *n* – Number of measurements used for calibration curve; *p* – Number of measurements used to obtain the concentration of the sample; *c* – Analyte concentration in the unknown sample, mol/l; *m*– Average of standard solutions, mol/l; S_{XX} - " $(ci - m)^2$; *Yj* – Analytical signal of the measurement j; *j* – Index for the number of measurements made in order to obtain the calibration curve; *i* – Index for the number of solutions for the calibration; *b* – Calibration curve slope, l/mol; *a* – Calibration curve intercept

If an equation of calibration curve has the form mentioned in Eq. (3):

$$\mathbf{Y = 3224x - 0.013} \qquad (3)$$

Where, *Y* – Analytical signal, absorbance; *x* – Analyte concentration, mol/l

The concentration C_{10} is obtained from the calibration curve equation. (Sample solution was measured ten times (p =10), the number of measurements made for obtaining the concentration)

Uncertainty of the liberation of 10 ml volume of 10 ml volumetric flask

Uncertainty in case of repeatability of the liberation of 10 ml volume of 10 ml volumetric flask was determined by filling up and weighing a 10 ml volumetric flask with standard solution.

Uncertainty associated with the sample mass m_{sample}

The sample mass was determined using the weight difference between the mass of weighing glass with and without sample.

RESULTS AND DISCUSSION

All the uncertainty sources were identified by using cause-effect diagram, and these sources are evaluated and measured.

Uncertainty due to concentration, C_{10}

Eq. (3) explains calibration curve, and Fig. 2 justifies the overlay spectra of standard solution and Fig. 3 justifies the calibration curve equation. Results of the linear regression are shown in Table 1. Ten repeated measured values of sample solution were used to determine analyte concentration C_{10} which are given in Table 2 and spectrum of sample solution is displayed in Fig. 4. Realizing the average of standard solution, we obtained average of the values 1.45×10^{-4} knowing that equation of calibration curve is parameters of regression curve was identified such as, slope 3224 and intercept 0.013. For the determination of calibration curve, 6 solutions were measured three times (total number of measurements is n=18). Thus:

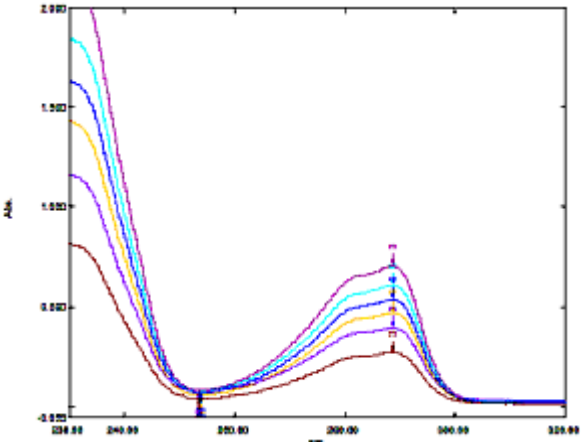

Fig. 2: *Overlay spectra of the standard solutions*

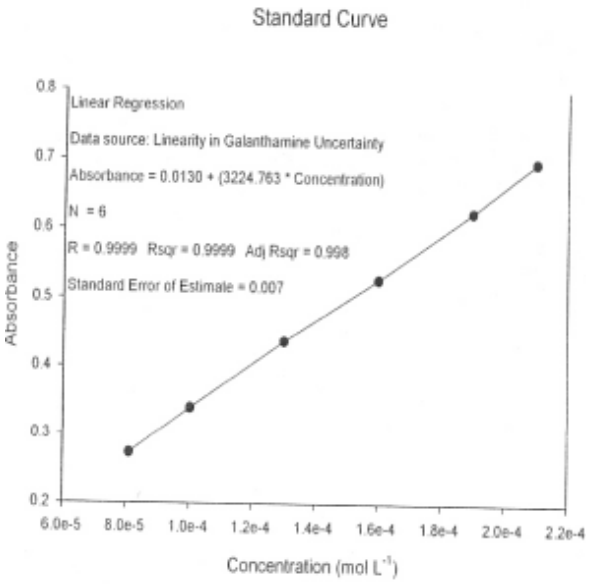

Fig. 3: *Calibration curve and linear regression analysis result*

Table 1: *Calculated parameters of the linear regression analysis*

S. No.	Statistic parameter	Value
1	Regression line equation	$Y = 3224x - 0.013$
2	Slope of the regression line	3224
3	Standard deviation of the slope, s^b	0.002702
4	Intersection to the origin, a	0.013
5	Standard deviation of intersection, s^a	0.018493
6	Correlation coefficient, R^2	0.9999
7	Relative standard deviation, RSD	0.011
8	Number of freedom degrees, n	5

Table 2: *Experimental results for concentration C_{10}*

S. No.	Conc. Found in Tablet (mg)	C.mol L^{-1}
1	0.0284	7.7×10^{-6}
2	0.0275	7.47×10^{-5}
3	0.0283	7.68×10^{-5}
4	0.0295	8.01×10^{-5}
5	0.0286	7.77×10^{-5}
6	0.0294	7.98×10^{-5}
7	0.0293	7.96×10^{-5}
8	0.0281	7.63×10^{-5}
9	0.0288	7.82×10^{-5}
10	0.0294	7.98×10^{-5}
Average	0.02873	7.76×10^{-5}

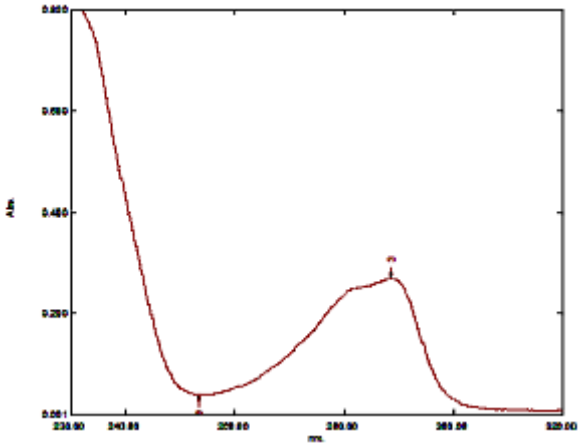

Fig. 4: *Zero order Spectra of galantamine tablets*

$$s_{xx} = 5.423 \times 10^{-10} \text{ mol/l} \quad (4)$$

$$s_r = 1.72 \times 10^{-2} \text{ mol/l} \quad (5)$$

$$u(C_{10}) = 3.6 \times 10^{-5} \text{ mol/l} \quad (6)$$

In conclusion, uncertainty for sample was C_{10} = 7.76×10^{-5} while $u(C_{10})$ = mol/l, so the standard relative uncertainty was:

$$\frac{u(C_{10})}{C_{10}} = 4.3 \times 10^{-1} \quad (7)$$

Uncertainty of the liberation of 10 ml volume of 10 ml volumetric flask

The volume of 10 ml volumetric flask is varied by three major parameters, (a) calibration at the time of manufacturing of volumetric flask, (b) repeatability and (c) temperature.

Calibration at the time of manufacturing of volumetric flask (claimed by manufacturer)

Deviation value from the nominal volume for 10 ml volumetric flask is ± 0.001 ml (at 27°C) given by manufacturer. If we assume that standard deviation is not claimed by manufacturer with confidence interval limit, standard value of uncertainty can be calculated with triangular distribution. Thus, uncertainty associated with liberation of 10 ml volume of 10 ml volumetric flask due to calibration u (V_{10}_cal) is,

$$u\left(V_{10\,cal}\right) = \frac{0.001}{\sqrt{6}} = 4.08 \times 10^{-4}\,\mathbf{mL} \qquad (8)$$

Repeatability

After filling and weighing of 10 ml volumetric flask, standard uncertainty of volumetric flask was established at 0.01 ml, due to repeatability u (V_{10}_rep).

Temperature

The manufacturer has calibrated volumetric flask at the time of manufacturing at a temperature of 27 °C, while temperature at laboratory varied with Ät = ± 4 °C. This difference can be overcome by calculating uncertainty value with estimation of temperature range and volume expansion coefficient. Volume expansion of liquid was taken into consideration as it is quite higher than expansion of volumetric flask. The volume expansion coefficient, ë, of water is 2.1×10^{-4} °C^{-1}. Thus uncertainty for 10 ml volumetric flask ÄV_{10} is:

$$\Delta V_{10} = V_{10} \times \gamma \times \Delta t \qquad (9)$$

Where: Ä V_{10} – Uncertainty of the 10 ml volumetric flask; V_{10} –Volume of the 10 ml volumetric flask; *ã* – Volume expansion coefficient; *Ät* – temperature variation in the laboratory.

Thus, we obtain an uncertainty for 10 ml volumetric flask of 0.0084 ml. Assuming temperature variation is a rectangular distribution, standard uncertainty for 10 ml volumetric flask due to the temperature effect will be u (V_{10}_temp):

$$u\left(V_{10_{temp}}\right) = \frac{4 \times 2.1 \times 10^{-4} \times 10}{\sqrt{3}} = 0.0048\,\mathrm{mL} \qquad (10)$$

Thus, standard uncertainty due to liberation of 10 ml volume of 10 ml volumetric flask will be:

$$u(V_{10}) = \sqrt{\left(u(V_{10-cal})\right)^2 + \left(u(V_{10-rep})\right)^2 + \left(u(V_{10-temp})\right)^2} \qquad (11)$$

$$u(V_{10}) = 0.011\ \mathbf{mL}$$

The standard uncertainty will be:

$$\frac{u(V_{10})}{V_{10}} = 1.1 \times 10^{-3}\ \mathbf{mL} \qquad (12)$$

Uncertainty associated with the sample mass m_{sample}

Estimation of analyte mass has three types of uncertainty sources such as sensitivity, linearity, and repeatability. Mass of the sample was expressed in kg to assure traceability of results.

Sensitivity

The weighed mass was of short range of difference and which was measured on same weighing balance. Thus sensitivity can be neglected.

Linearity

Data from manufacturer indicated a linearity value is 0.0001 g. To determine uncertainty value standard uncertainty was considered. A rectangular distribution was assumed to convert contribution of linearity. Contribution of linearity needed to be considered twice in the determination of standard uncertainty (for tare and for analyte mass):

$$u = \frac{0.0001 \times 10^{-3}}{\sqrt{3}} = 5.78 \times 10^{-8}\,\mathbf{Kg} \qquad (13)$$

Repeatability

Uncertainty associated with repeatability is 0.0002 g. In conclusion, uncertainty due to sample mass u (m_{sample}) is:

$$u\left(m_{sample}\right) = \sqrt{2 \times (5.78 \times 10^{-8})^2 + (0.0002 \times 10^{-3})^2} = 4.6 \times 10^{-14}\,\mathrm{Kg} \qquad (14)$$

The relative uncertainty due to sample mass is:

$$\frac{u(m_{sample})}{m_{sample}} = \frac{4.6 \times 10^{-14}}{2.873 \times 10^{-5}} = 1.6011 \times 10^{-9} \qquad (15)$$

Quantity of GH in tablets, expressed as mol/kg, was calculated using Eq. 1. Thus, we obtain a quantity of 2.7×10^{-5} mol/kg. Table 3 displays the intermediate values and their standard uncertainties. To calculate composed uncertainty of sample quantity (Q_{sample}), standard uncertainty is calculated using following equation:

$$\frac{u\left(Q_{sample}\right)}{Q_{sample}} = \sqrt{\left(\frac{u(V_{10})}{V_{10}}\right)^2 + \left(\frac{u(C_{10})}{C_{10}}\right)^2 + \left(\frac{u(m_{sample})}{m_{sample}}\right)^2} \qquad (16)$$

Thus:mol/kg, $u(Q_{sample}) = 1.16 \times 10^{-5}$ mol/kg

As we consider the confidence level of 95 % and a coverage factor k=2, $u(Q_{sample})$ the will be as follows as by equation of coverage factor **expanded uncertainty** $U = k\,u_c$, mol/kg

$$u(Q_{sample}) = 2 \times 1.16 \times 10^{-5} = 2.32 \times 10^{-5}\ \text{mol/kg} \qquad (17)$$

The cause-effect diagram for analysis of GH is explained in Fig. 5. The contribution of different parameters and their influence to uncertainty are given in Fig. 6.

Table 3: *Summary of contribution of the measurement to uncertainty for determination of GH from tablets through UV-V is Spectrometry*

Sl. No.	Parameter	Value	Standard uncertainty, u(x)	Relative standard uncertainty, u(x)/x
1	Volume, V_{10} (mL)	10	1.1×10^{-2}	1.1×10^{-3}
2	Sample concentration, C_{10} (mol L^{-1})	7.76×10^{-5}	3.6×10^{-5}	4.3 x 10^{-1}
3	Mass sample, m_{sample} (Kg)	2.873×10^{-5}	4.6×10^{-14}	1.6011×10^{-9}

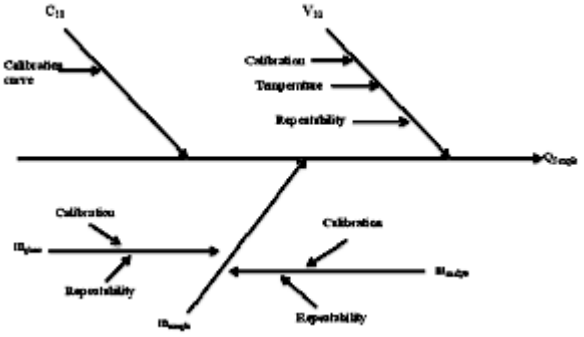

Fig. 5: *Cause-effect diagram for the analysis of GH*

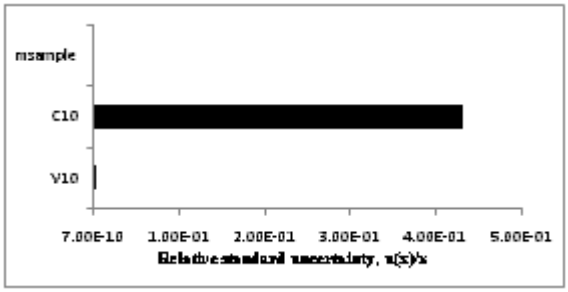

Fig. 6: *Contribution of the different parameters*

CONCLUSION

Thus with help of a cause-effect analysis, it is possible to measure uncertainty in the determination of GH from pharmaceutical formulations through ultraviolet spectrophotometry. Determination of various components of uncertainty is a best method to confirm that results obtained of analytical methods are certain. It is concluded from the present study that uncertainty is influenced by sample concentration rather than liberation of 10 ml volumetric flask and sample mass. Thus sample concentration is the major factor to achieve precise results of analysis.

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