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ANALYSIS FOR EPHEDRINE ALKALOID UTILIZING ISOCRATIC HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A method to assay ephedrine hemihydrate (ephedrine alkaloid), which has various biological activities, is presented using isocratic high performance liquid chromatography (HPLC). Elution of the analyte is detected with ultraviolet light detector, set at 255 nm. The stock solution of this ephedrine alkaloid was prepared in solvent conditions that consisted of 10% ethanol (v/v) and 90% water (v/v), at a concentration of 7.1568×10^{-2} molar. The test samples of ephedrine hemihydrate that were injected into HPLC instrument were mainly in a solvent that consisted of 90% (v/v) water and 10% ethanol (v/v). The solvent utilized for column of the HPLC instrument was 5% ethanol, 1% glacial acetic acid (v/v), and 94% water (v/v). The limit of detection (LOD) was found to be 2.9833×10^{-5} molar and the limit of quantitation (LOQ) was found to be 8.9500×10^{-5} molar. A standard curve presented showed a coefficient of determination of $R^2 = 0.9857$, which indicates that the model describes 98.57% variance in the dependent variable (peak area) that is predictable from the independent variable (molar concentration). The Pearson r correlation coefficient of this standard curve is 0.9928, indicating very high positive correlation. Ephedrine hemihydrate is assayed from matrixes that are utilized in the administration of this drug, including water and cellulose for tablet formulation. The determination of the drug ephedrine alkaloid is consistent and accurate utilizing isocratic conditions with HPLC.

KEYWORDS: Ephedrine alkaloid, HPLC, isocratic, ephedrine hemihydrate.

INTRODUCTION

Ephedrine (ephedrine alkaloid) occurs naturally in various types of plants, but has been prepared synthetically in 1927.[1] Ephedrine is one of six compounds of this type derived from the plant *Ephedra sinica*.[1] It is a sympathomimetic drug (stimulant compounds which mimic the effects of endogenous agonists of the sympathetic nervous system) that has been utilized as nasal decongestant, for bronchospasms, for obesity, and as a pressor agent (elevates arterial blood pressure).[1] This compound can be administered orally and has been noted to have a physiological action similar to adrenaline.[2] Ephedrine is known to enable short-term weight and fat loss.[3] Other patient focused clinical application is for allergies, hay fever, asthma, and bronchitis associated with flu and swine flu.[4]

Some clinical outcome have revealed that ephedrine use does show side effect, some of which suggest significant safety hazards.[5] These side effects have been shown to involve cardiovascular effects, including the following observations: hypertension, myocardial infarction, seizures, and strokes that can be fatal.[5] Other studies have revealed that consumption of ephedra based supplements have been shown to result in cardiac effects intracerebral hemorrhage, mania, nephrolithiasis (crystalline stones (calculi) within the urinary system including kidneys and ureter), and death.[6]

Methods for identification and assay of ephedrine type alkaloid content include high performance liquid chromatography.[7] Other methods evaluated include column-switching cation exchange high performance liquid chromatography (HPLC).[8] Another investigator showed that high performance liquid chromatography with ultra-violet detection can identify ephedrine.[9] A titrimetric method for determination of ephedrine utilizes methyl red indicator in solution that has been acidified, then titrated with sodium hydroxide standard to quantify the amount of excess acid.[10] Another titration method for ephedrine hydrochloride was based on the formation of a copper complex formation.[11] The methodology presented here is shown to use high performance liquid chromatography with ultra-violet detection that is effective and useful for industrial quality control, compound verification, and drug assay.

MATERIALS AND METHODS

Chemical Reagents

Solvents were analytical grade and obtained from Sigma-Aldrich (St. Louis MO 63178 USA). The ephedrine alkaloid drug (ephedrine hemihydrate) to prepare test

samples was obtained from Merck and Company Inc. (Rahway, New Jersey USA). Distilled water is utilized wherever the use of aqueous solvent is noted.

Instrumentation

An Alltech 426 HPLC Pump and Linear UVS 200 detector were used for high performance liquid chromatography analysis (Deerfield, Illinois 60015-1899). Reversed-phase isocratic conditions (unchanging column solvent) are applied for all types of samples injected into HPLC injector. The HPLC Column consists of 5µ packing, having a length of 150 millimeters, and an internal diameter of 4.6 millimeters. Ultraviolet detection is at a wavelength of 255 nm.

HPLC Instrument Settings

For this HPLC analysis, a reversed-phase C-18 octadecylsilyl (C18H37) bonded phase column packing was utilized throughout the study. Detection of eluted species was accomplished by use of ultraviolet detector set to 255 nm, rise time 0.1, and with range AUFS set to 1.0. The HPLC pump was set to 1300 psig with one milliliter per minute flow rate. The actual volume injected into the column is 20 microliters. The dead time for the elution of non-retained species is 1.5 minutes and is calculated by the relationship, dead time= volume/flow rate = 1.5 mL/1.0 mL/min. Dead volume (V = 0.01 (length of column)) is 1.5 mL. The column solvent used throughout the study is as follows: 52.6 mL of 95% ethanol, 10 mL of glacial acetic acid, and 937.4 mL of distilled water.

Preparation of Samples

Column solvent utilized is as follows: total volume of 1000 mL prepared by adding 52.6 mL of 95% ethanol, 937.4 ml of distilled water, and 10 mL of glacial acetic acid (stock of glacial acetic acid at 17.4 molar). Therefore, the working concentrations will be 5% ethanol, 0.174 molar acetic acid, and 93.7% water (v/v). Sample solvent used for solubilizing ephedrine alkaloid for assay: 95% distilled water with 5% ethanol. Stock standard of ephedrine alkaloid (ephedrine hemihydrate molecular weight 174.24 grams/mole) was prepared by dissolving 3.1175 grams of the compound into 250 mL volumetric flask of 10% ethanol (v/v) in distilled water, making a mixture of 7.1568×10^{-2} molar. If any sample required clarification prior to HPLC analysis, this was accomplished by Whatman 6900-2502 GD/X Sterile Syringe Filter, 25 mm, 0.2 Micron, PVDF Filtration Medium, with a suitable plastic syringe. Samples for HPLC analysis were in sample solvent. Oral administration is achieved in water, therefore, samples in water only were analyzed for evaluation. Tablet/solid samples were the drug prepared in various known percentage of combinations of excipient cellulose.

Statistical Analysis

Where indicated the numerical analysis utilizing Paired tests, F and T test, Kruskal-Wallis, and correlation between sets of data is accomplished by PAST version 2.06 (copyright Hammer and Harper 1999-2011). Microsoft EXCEL (copyright 2010 Microsoft Corporation, Microsoft Office Professional Plus 2010) and PAST v. 2.06 also performed summary statistical analysis.

Detection of numerical outliers was accomplished by use of the Grubb's test for outliers (or extreme studentized deviate) was performed by Graph Pad InStat version 3.00 (Copyright 1992- 1998 Graph Pad Software Inc. (www.graphpad.com) for Windows 95, San Diego California USA). EXCEL and PAST v. 2.06 accomplished linear regression. Passing-Bablok analysis was accomplished by ACOMED statistik: (www.acomed-statistik.de, copyright: Dr. Thomas Keller). The Bland-Altman plot (also referred to as difference plot) was accomplished by use of Method Validator (www.multiqc.com).

RESULTS AND DISCUSSION

Ephedrine alkaloids (from family *Ephedraceae*) are considered to be botanical dietary supplements that contain sympathomimetic stimulants.[8] Commercially they are sold as body building supplements and/or weight loss additions to diet. However, when these compounds are consumed in addition to caffeinated products then adverse cardiovascular and central nervous system effects have been noted, even causing death.[8]

Because of the potential destructive effects of consumption of ephedrine alkaloids, a method for assay would be practical and useful to monitor presence of this compound. In addition, methods for determination of ephedrine would be useful for monitoring of quality control within industrial synthesis, verification of content for drug formulations, contamination monitoring, and patient compliance of dosage form and amount.

The molecular structure of ephedrine alkaloid (2-(methylamino)-1-phenylpropan-1-ol hemihydrate) is presented in Figure 1. At formula weight of 174.24 grams/mole, it is considered as a small molecule drug.[2] The molecular scaffolding includes an aromatic ring, secondary amine group, and secondary hydroxyl group.

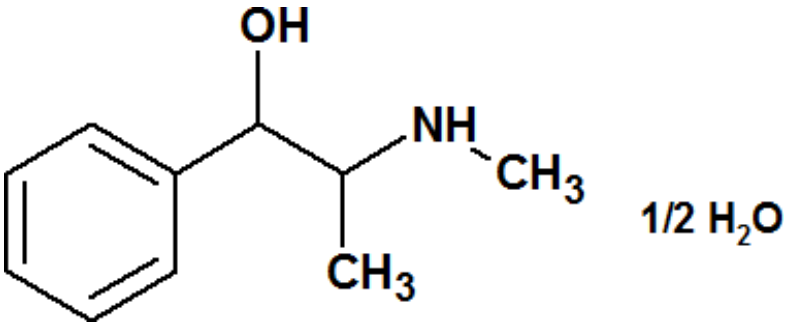

Figure 1: Molecular structure of ephedrine alkaloid (2-(methylamino)-1-phenylpropan-1-ol hemihydrate). Having molar mass 174.24 grams/mole. The structure possesses an aromatic ring and secondary amine group.

To accomplish the HPLC assay of ephedrine alkaloid utilizing ultraviolet (UV) light detection it is useful to first determine the wavelength of light in which this drug has the highest absorbance and set the UV detector to that specific wavelength. This was accomplished for this methodology with the absorbance spectrum shown in Figure 2. Examining a wavelength spectrum reaching from 230 nm to 300 nm in a solvent having ethanol and water, it was found that the maximum absorbance for the drug is attained at 255 nm.

A broad absorbance area was observed from 230 nm to 280 nm, which peaked at 255 nm for this assay methodology. Property of absorbance is determined and registered by the HPLC instrumentation in terms of peak area, having units of Area uV. Min. Conversion to peak area by the instrument processor allows for higher resolution of detector response and useful comparison among mixtures with comparatively large or slight differences in analyte presence.

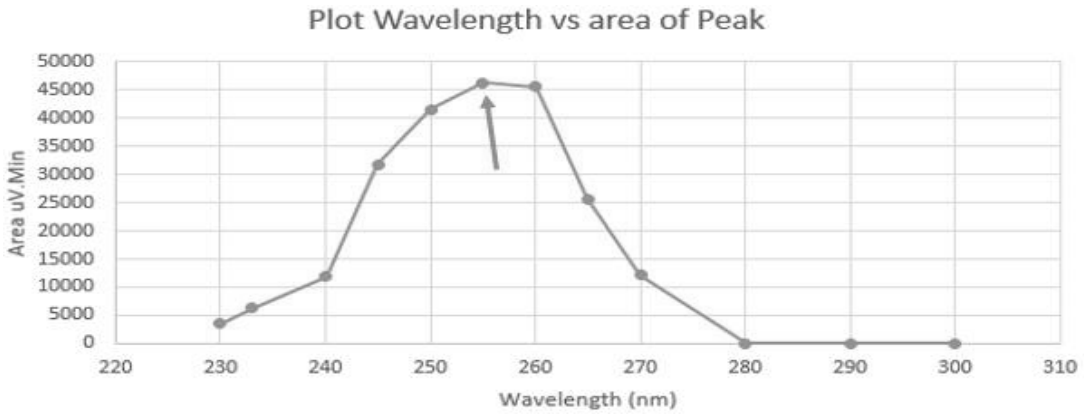

Figure 2: Absorbance spectra of ephedrine alkaloid from 300 nm to 230 nm. The optimum absorbance value occurs at 255 nm. Mixture is 0.04497 molar ephedrine alkaloid in 10% ethyl alcohol (v/v) and 90% distilled water.

The column dead time peak (t_o) is the time it takes for a compound to go through the column while not interacting with the column.[12] A corresponding dead volume (or hold-up volume), V = 0.01(length of colume), is the volume of mobile phase inside the column. This volume comprises both the volume of mobile phase between the packing particles (the interstitial volume) and the volume within the particles (the pore volume).[12]

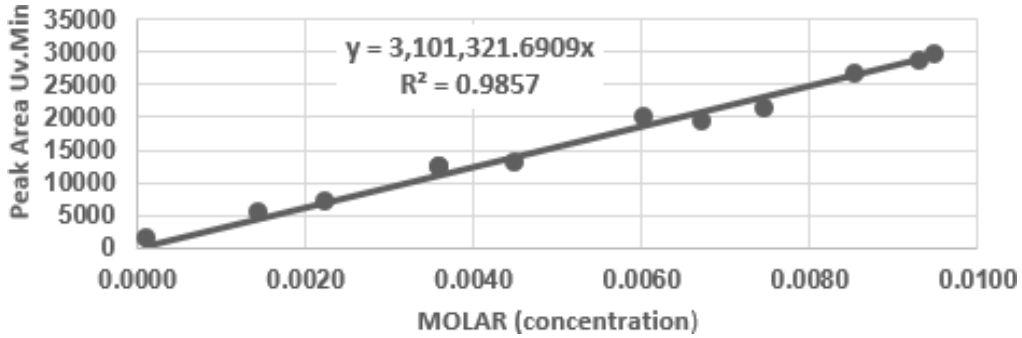

Figure 3: Standard curve for determination of ephedrine alkaloid. Equation of line is y = 3,101,321.6909 x, with coefficient of determination at 0.9857, indicating that molar concentration accounts for 98.57% of the variance in peak area. Pearson r correlation is 0.9928 indicating very strong positive relationship.

For this study, the dead time is 1.5 minute and dead volume is 1.5 mL. Generally, the ephedrine alkaloid analyte eluted at approximately 6.2 minutes.[12,13] It is generally accepted that the limit of detection (LOD) for HPLC analysis is that concentration of analyte detected at three times the viewed noise level,[13,14] (LOD) in this study determined to be 2.9833×10^{-5} molar.

The limit of quantitation (LOQ) is determined to be 10 times the baseline,[13,14] LOQ was determined to be 8.9500×10^{-5} molar.

Table 1: Percent Recovery for Ephedrine Alkaloid by HPLC.

Calculated Molar Concentration	HPLC Assayed Molar Concentration	Percent Recovery
2.2365×10^{-3}	2.3792×10^{-3}	106
3.1311×10^{-3}	3.0088×10^{-3}	96.0
5.3676×10^{-3}	5.6843×10^{-3}	105
9.3038×10^{-3}	9.2260×10^{-3}	99.2
6.0833×10^{-3}	6.1995×10^{-3}	101
6.7095×10^{-3}	6.3214×10^{-3}	94.0
9.4828×10^{-3}	9.5882×10^{-3}	101
7.2463×10^{-3}	7.6639×10^{-3}	105
8.1766×10^{-3}	7.7459×10^{-3}	95.0
8.5345×10^{-3}	8.5891×10^{-3}	100
5.6002×10^{-3}	5.8572×10^{-3}	104
7.3894×10^{-3}	7.0153×10^{-3}	95.0
5.3676×10^{-3}	5.6356×10^{-3}	104
5.3676×10^{-3}	5.2805×10^{-3}	98.4
4.4730×10^{-3}	4.3014×10^{-3}	96.0
4.7772×10^{-3}	5.0729×10^{-3}	106
5.8507×10^{-3}	5.9151×10^{-3}	101
3.3458×10^{-3}	3.3942×10^{-3}	101
7.8188×10^{-3}	7.4644×10^{-3}	95.5
8.1766×10^{-3}	7.7207×10^{-3}	104
5.2424×10^{-3}	5.2466×10^{-3}	100
5.9580×10^{-3}	6.0357×10^{-3}	101
6.6737×10^{-3}	6.6250×10^{-3}	99.3
7.2105×10^{-3}	7.3016×10^{-3}	101
7.5683×10^{-3}	7.5254×10^{-3}	99.4
8.3556×10^{-3}	7.9227×10^{-3}	95.0

The nearness between the expected concentration value and the value found following HPLC determination is expressed by calculating the percent recovery of analyte recovered.[13,14]

The percent recovery following ephedrine alkaloid injection gave results presented in Table 1. The calculated values of molarity showed a mean of 6.3634×10^{-3} molar with median of 6.3785×10^{-3} molar. The HPLC assayed recovery values mean is 6.3354×10^{-3} molar with a median of 6.2604×10^{-3} molar. The average percent recovery is 100% with standard deviation of 3.7%.

The paired tests for calculated and HPLC assay molarities indicated that both populations have the same mean (P=.58) and the same median (P=.85).[15] The Pearson r correlation of these two groups of molarities is 0.9913, indicating a very strong positive correlation. One-way ANOVA analysis of calculated and HPLC assayed molarities also indicated equal means (P=.96).[15] The Kruskal-Wallis and Mann-Whitney tests indicated the two groups have equal medians, (P=.99) and (P=.99), respectively.[15] The coefficient of variation (CV) is a measure of relative variability.[15] The CV for calculated and HPLC assayed values are 29.48 and 28.45, respectively. There is less variation in the HPLC assayed values than for calculated values.

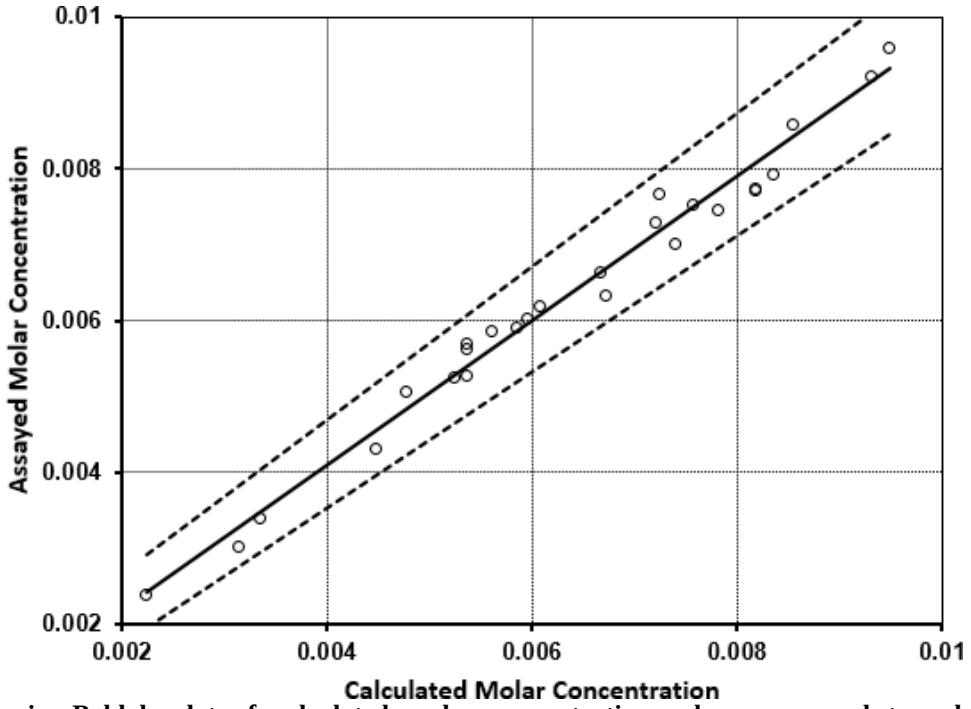

Figure 4: Passing-Bablok plot of calculated molar concentration values compared to values of molar concentration values obtained from assay by HPLC. Value of 1 is contained within the 95% confidence interval of the slope (0.8970 to 1.0113). The value of 0 is contained within the 95% confidence interval of the y-axis intercept (0 to 0.007). Pearson r correlation is 0.9913, with no departure from linearity by applying "runs test" (*P*=.08).

Method comparison can be used to determine if the HPLC method of measurement is equivalent to the expected calculated values of measurement in use.[16] Linear regression, when applied to comparison of data from such as HPLC assay to calculated (expected), provides useful information about proportional, constant, and random error.[16] Passing and Bablok regression proposes a linear regression procedure with no special assumptions regarding the distribution of the data and is a method is based on ranking the observations.[16]

For Passing-Bablok plot shown in Figure 4 the 95% confidence interval of the slope is 0.8970 to 1.0113 (slope equal to 0.9538). The 95% confidence interval for the y-axis intercept is 0.0000 to 0.0007 (y-axis intercept equal to 3.000×10^{-4}). The confidence interval for the y-axis intercept includes zero and the confidence interval for the slope includes the value of 1. Therefore, there are no constant differences between the actual molarity and the HPLC measured molarity, in addition, these values can be used interchangeably.[16] Therefore, the calculated molar values are representative of the HPLC assayed measured molar values.[16] The correlation coefficient of the line (see Figure 4) is 0.9913, with a coefficient of determination R^2 of 0.9827. The runs test indicated that there is no significant departure from linearity.[15]

Ephedrine alkaloid can be introduced as an aqueous mixture for oral administration, but can be processed within hard tablets, also for oral administration.[7] This methodology can assay for this drug following the solubilizing of cellulose-type tablets containing ephedrine alkaloid.

Solubilizing of tablets is done in solvent with 10% ethanol (v/v) and 90% water (v/v). The mixture can be filtered using Whatman sterile syringe filter cartridge. Samples were then assayed similarly to other described in Materials and Methods. The amount of cellulose within tablet form having ephedrine alkaloid at about 4.4693×10^{-3} molar, varied significantly to include amounts such as: 0.027 grams, 0.005 grams, 0.052 grams, 0.043 grams, etc. The HPLC was able to identify the ephedrine alkaloid eluting and concentrations all within an alpha of 0.05 two-sided and with no outliers, by Grubb's test (extreme studentized deviate) with (*P*=.05).

This drug can be introduced in aqueous beverages or similar, for oral administration. Likewise as above various samples of this drug were prepared in straight aqueous solvent and assayed utilizing the conditions described in Materials and Methods. The concentration of ephedrine alkaloid was maintained at approximately 4.6788×10^{-3} molar. The HPLC analysis again determined the amount of the drug consistently within an alpha of 0.05 two-sided and with no outliers by the Grubb's test with (*P*=.05). HPLC assay is effective for both aqueous derived samples of this drug and solid tablet consisting of cellulose excipient.

Ephedra has been used for athletic enhancement, for weight loss, and obesity. It is also used for allergies and hay fever; nasal congestion; and respiratory tract

conditions.[7] Methods for assay of this compound are needed for a variety of applications, to include: quality control at industrial processing level, patient compliance, drug verification, and investigative examination.

Techniques to assay drugs utilized for human consumption are needed to maintain effective monitoring for whatever administration and clinical use appears.

CONCLUSION

The sympathomimetic drug ephedrine alkaloid was assayed by HPLC from solvent mixtures consisting of ethanol and water. The UV maximum absorbance of this small molecule drug occurred at 255 nm when examined between 230 nm and 300 nm. The compound ephedrine alkaloid was successfully assayed from matrix form that includes excipient cellulose (for tablet formulations) and aqueous mixture (for drink form of delivery). The limit of detection (LOD) is determined to be 2.9833 x 10^{-5} molar and the limit of quantitation (LOQ) was 8.9500 x 10^{-5} molar. The standard curve showed a Pearson r correlation coefficient of 0.9928, equation y = 3,101,321.6909(x), with coefficient of determination at 0.9857. This methodology would be useful for quality control monitoring in industrial manufacturing and drug composition verification.

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REFERENCES

1. Goodman LS, Gilman A. The Pharmacological Basis of Therapeutics. 4th ed., London; The MacMillan Company, 1970.
2. Saluja M, Kumar S, Agarwal. Advanced Natural Products. Meerut; Krishna Prakashan Media, 2014.
3. Shekelle PG, Hardy ML, Morton SC, Maglione M, Jojica WA, Suttorp MJ, Rhodes SL, Jungvig L, Gagne J. Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta analysis. JAMA, 2003; 289(12): 1537-45.
4. Backer R, Tautman D, Lowry S, Harvey CM, Poklis A. Fatal ephedrine intoxication. J Forensic Scie, 1997; 42(1): 157-9.
5. Persky AM, Berry NS, Pollack GM, Brouwer KLR. Modelling the cardiovascular effects of ephedrine. Br J Clin Pharmacol, 2004; 57(5): 552-62.
6. Wahl MC, Grant C, Jesperson R. Acute psychosis from a dietary weight control supplement, J Toxicology: Clinical Toxicology, 2000; 5(38): 522-29.
7. Gurley BJ, Wang P, Gardner SF. Ephedrine-type alkaloid content of nutritional supplements containing ephedra sinica (Ma-Huang) as determined by high performancd liquid chromatography, 1998; 87(12): 1547-53.
8. Niemann RA, Gay ML. Determination of ephedrine alkaloids and synephrine in dietary supplements by column switching cation exchange high-performance liquid chromatography with scanning-wavelength ultraviolet and fluorescence detection. J Agricultural and Food Chem, 2003; 51: 5630-38.
9. Roman MC. Determination of ephedrine alkaloids in botanicals and dietary supplements by HPLC-UV. J AOAC INT, 2004; 87(1): 1-14.
10. Marques MRC. Titrimetry, Pharmacopeial Forum, 2018; 44(4): 3050.
11. Medvedovskii AQ. Titration method of determination of ephedrine hydrochloride on the basis of copper complex formation. Farm Zh, 1971; 26(5): 70-3.
12. Dolan JW. Column dead time as a diagnostic tool. LCGC North America, 2014; 32(1): 24-9.
13. Shabir GA. Validation of HPLC methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the U.S., FDA, USP and ICH. Journal of Chromatography A, 2003; l987: 57-66.
14. Shrivastava A, Gupta VB. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles of Young Scientists, 2011; 2(1): 21-5.
15. Davis JC. Statistics and Data Analysis in Geology. New York; John Wiley & Sons: 1986.
16. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. application of linear regression procedures for method comparison studies in clinical chemistry, part I. J Clin Chem Clin Biochem, 1983; 21: 709-20.