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Pharmaceutical formulations with Spectrophotometric Determination of Bromhexine HCl

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Abstract

Theophylline has been used as an internal standard in the development of a quick, easy, cheap, and sensitive liquid chromatographic method for the measurement of gemcitabine in injectable dose forms. With a mobile phase made up of 90% water and 10% acetonitrile, chromatographic separation was accomplished on a Phenomenex Luna C-18 column (250 mm 4.6 mm; 5) (pH). Gemcitabine and theophylline signals were captured at 275 nm. The 0.5 to 50 g/mL concentration range was covered by linear calibration curves. As high as 0.999 was the correlation coefficient. 0.1498 and 0.4541 g/mL, respectively, were the limits of detection and quantitation. Less than 2% were within- and between-day precision. From 100.2% to 100.4% of the method's measurements were accurate. The medication was stable to sunshine and UV light, according to stability experiments. Under alkaline stress, the medication yields six distinct hydrolytic compounds, while under acidic stress, it yields three. The medicine is also degraded by oxidative and aqueous stresses. Comparing the alkaline condition to other stress settings, degradation was greater in this one. Using experimental design, the robustness of the approaches was assessed. According to validation, the suggested approach is suitable for the quantitative analysis and is specific, accurate, precise, reliable, robust, and reproducible.

For the analysis of the medication in pharmaceuticals, five spectrophotometric techniques for bromhexine HCl determination have been devised, verified, and put to use. The three triphenylmethane dyes—bromothymol blue (BTB), bromophenol blue (BPB), and bromocresol green—are used to ion-pair complex the medication in the methods A, B, and C. (BCG). The complexes are extracted into chloroform, and as a function of drug concentration, absorbance is measured at about 415 nm. In each instance, it is discovered that the complex's stoichiometry is 1 : 1. The charge-transfer complexation of the neutralised drug with the iodine in Method D outcome in the production of the iodide ion, whose absorbance at 366 nm is determined as a function of the drug concentration. this complex's composition is also 1: 1. The concept behind Method E is the oxidation of the medication with alkaline KMnO4, which produces a green manganate ion with a maximum wavelength of 610 nm. The reaction's kinetics is observed as the intensity of the green hue grew with time, and calibration curves are built using the initial rate and fixed time approaches. The methods can be utilised successfully in industries for the assay of drugs in pure form and pharmaceuticals, according to excellent recovery studies with high accuracy and precision.

Keywords: Pharmaceuticals; Bromophenol

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Introduction

A -difluoronucleoside, purine antimetabolite, gemcitabine hydrochloride is 4-amino-1-[(2R, 4R, 5R)-3, 3-difluoro-4-hydroxy-5-(hydroxymethyl) oxolan-2-yl] pyrimidin-2-one. The medication is an anticancer agent that is frequently used to treat a variety of human cancers, including ovarian, lung, pancreatic, bladder, urothelial, and breast cancer [1]. Presently, it is sold as a lyophilized powder. The medication is also widely used as an immunosuppressive, antiviral, enzyme inhibitor, and radiation sensitizer. Gemcitabine is a prodrug that is transported into cells by nucleoside transporters. It then undergoes an intracellular conversion to its diphosphate and triphosphate counterparts, which is completed by deoxycytidine kinase. By blocking thymidylate synthetase, which prevents DNA synthesis and chain elongation, the triphosphate derivative gets absorbed into the DNA strand, adding to the drug's anti-cancer effects. The diphosphate derivative prevents ribonucleotide reductase, an enzyme that catalyses the production of the deoxynucleoside-triphosphate needed for DNA synthesis, from doing its job. Endogenous nucleoside triphosphate and gemcitabine triphosphate compete for DNA synthesis [2]. The verified stability indicating approach that can separate the hydrolytically degraded product of gemcitabine has been published for the separation and identification of gemcitabine's degraded products under acidic stress conditions. To the best of our knowledge, none of the HPLC methods, however, reported the gemcitabine oxidatively damaged product [3]. Previous formulation methods described in the literature are less reliable and require additional research for method development and validation. In order to prove their specificity, stability-indicating methods must assess the medicine in the presence of its degradation products. The current study proposes an RP-HPLC method for the detection of gemcitabine in dosage forms that is straightforward, quick, accurate, precise, and strong stability suggesting. Using a 24 factorial design, the robustness of the approach was investigated [4].

Its chemical name is 2-amino-3,5-dibromo-N-cyclohexyl-N-methyl benzenemethanamine hydrochloride. Bromhexine HCl (BRH) is a mucolytic drug used to treat respiratory diseases brought on by viscid or profuse mucus. It is legal to use the medicine in IP and BP. Due to the drug's significance to human physiology, its chemical and physical characteristics have been used to quantify it [5]. Flow injection analysis with ion-selective electrodes, inductively coupled plasma mass spectrometry, electrokinetic chromatography, electrochemical oxidation at the glassy carbon electrode, liquid chromatography, liquid gas chromatography, GC with mass detection, and voltammetry are among the different analytical techniques used to quantify the drug as a single active pharmaceutical ingredient. Utilizing UV spectrophotometry, both direct and derivative, HPLC, and mixed formulations, the drug's dosage has also been determined [6]. These techniques require time-consuming experimentation and expensive, hardly available equipment. Based on the creation of chromophore by the interaction of the drug with an analytical reagent, such as chromogen, simple, accurate and precise procedures using spectrophotometry have also been developed. Pharmaceutical analysis has a lot of potential because there are so many analytical reagents that can be used as chromogens [7]. A thorough review of the literature revealed that the quantification techniques we are interested in have not yet been reported. As a outcome, in this communication, we report five quantification techniques, namely A, B, C, D, and E, that have been developed and validated for quantitative measurement of bromhexine HCl both in pure forms [8].

Material and Methods

Chemicals and Reagents [9]

Shilpa Medicare Limited, Raichur, India, provided the gemcitabine sample. Hetero Pharmaceutical Ltd., Hyderabad, India provided theophylline as a gift sample. The drug's marketed version was bought on the local market. Unless otherwise specified, all reagents were of analytical grade. Water of reverse osmosis and HPLC quality was used throughout. Panreac provided the methanol and acetonitrile.

HPLC Instrumentation and Conditions

The study used a Shimadzu Prominence high pressure liquid chromatographic instrument equipped with a Luna C-18 column (250 mm 4.6 mm; 5), an LC 20AT-VP solvent delivery system, a universal loop injector (Rheodyne 7725 I with a 20 L injection capacity, and an SPD 20A UV-visible detector (max 275 nm). Utilizing the LC-Solution programme, data was acquired. Using acetonitrilewater as the mobile phase, pH was adjusted to 7.0 using triethylamine and orthophosphoric acid. Chromatographic studies were performed. Every day, a 0.45 m membrane filter was used to prepare the mobile phase (Millipore Corp., USA). Column was kept at a constant temperature of 25 1.HPLC Instrumentation and Conditions.

Gemcitabine Standard and Working Solutions

Using an accurate scale, 100 mg of gemcitabine was put into a 100 mL calibrated flask and dissolved in the necessary amount of methanol. The void volume was then filled with methanol to create a stock solution with a concentration of 1000 g/mL. To create working solutions (25, 100, and 200 g/mL), the stock solution was further diluted with mobile phase.

Preparation of the Internal Standard (IS) Solution

Theophylline was put into a 100 mL volumetric flask after being precisely weighed, and 25 mL of methanol was used to dissolve it. The resulting solution was extensively sonicated until all of the medication was completely dissolved. Water was added to adjust the volume.

Preparation of Sample for Assay

The substance was included in twelve injectable vials of lyophilized powder from two different batches. They were stripped of their aluminium closures. Both with the medicine inside and empty were used to weigh the vials. The lyophilized powder's weight was determined using the information that was provided. It was transferred quantitatively to a 100 mL calibrated volumetric flask together with 10 mL of the internal standard solution and an

accurately weighed quantity of this powder equating to 10 mg of the medication. The flask was filled to volume with water after 50 mL of methanol was added. The mixture was then swirled, sonicated for five minutes, and completed.

Materials

In the investigation, HPLC grade chloroform, 1,2 Dichloroethane (DCE), Analytical grade (AR) HCl, Sodium acetate, KMnO4, Sodium hydroxide, and dyes, including (a) BTB (b) BPB (c) BCG, supplied by Sd Fine Chemicals, Mumbai, were utilised. Iodine (BDH, Poole, UK) was double sublimed and kept dry in a vacuum desiccator (mp 113.6-3°C). Freshly manufactured (daily) iodine in 1,2-dichloroethane (DCE) was created by combining 254 mg of solute with 50 mL of solvent (4.0 103 M). As a gift sample, the medication was obtained from Aurobindo Pharmaceuticals in Hyderabad.

Methods A, B, and C

The principles of the techniques A, B, and C are based on the drug's interaction with bromothymol blue (BTB), bromophenol blue (BPB), and bromocresol green (BCG), which outcomes in the formation of chloroform-extractable ion pair complexes that absorb at about 415 nm for each. Since the absorbance of this band increases with drug concentration, it served as the foundation for drug quantification. The dyestuffs were dissolved in doubly distilled water at a concentration of 0.025%. pH 2.8, 2.5, and 3.5 sodium acetate hydrochloric acid buffers were created by combining 50 mL of 1.0 M sodium acetate solution with 49.50 mL, 50.50 mL, and 46.25 mL of 1.0 M HCl solution, respectively. These mixtures were then diluted to 250 mL using doubly distilled water.

Method D Interaction of Iodine with Drug

The process is dependent on the interaction of the neutralised medication with iodine, which outcomes in the generation of an iodide ion with an absorption band at 366 nm. Since the absorbance of this band increases with drug concentration, it served as the foundation for drug quantification. The colour of the iodine changed from violet to light brown to pale yellow when the iodine solution made in DCE and BRH were combined. As a outcomes, a new band of light at 366 nm was visible in the absorption spectra. This is attributable to the ion that was created as a outcome of the drug's interaction with iodine.

Initial Rate Method E

A series of 10 mL standard flasks were filled with aliquots of bromhexine HCl solution containing 2.5 mg of medication per 1 to 8 mL. 1.0 mL of 0.45 M NaOH and 1 mL of 1 102 M potassium permanganate were added to each flask in turn before being diluted with distilled water at 25 1 C. Each flask's contents were thoroughly mixed before the increase in absorbance as a function of time was detected at 610 nm. By measuring the slope of the tangent to the absorbance-time plot, the beginning rate of the reaction (]) at various concentrations was calculated. The calibration graphs were created by graphing the starting rate of reaction (]) versus bromhexine HCl concentration.

Results

A number of factors, including the solvent, solvent strength, detection wavelength, flow rate, elution duration, asymmetry, and plate numbers, were taken into account in order to obtain the best separation possible. Gemcitabine hydrochloride and an internal standard were injected into several mobile phases of water, acetonitrile, or water, methanol, during optimization, and the retention time, tailing factor, and resolution factor were recorded. In certain mobile phases, the peak was deformed, while in other mobile phases, the compound eluted out fast, indicating a shorter retention period and, consequently, a less significant separation on the column. Gemcitabine's pKa is 3.5, making it unstable at acidic PHS. Various mobile phases with a pH of 7.0 were utilised. A appropriate mobile phase was chosen since it could separate the analytes: acetonitrile-water.

By contrasting the chromatograms produced from lyophilized powder, internal standards, and drug standards, the specificity of the approach was evaluated. There were no evidence of any coeluting peaks from the diluents, and the retention periods of the drug from standard solutions and from lyophilized powder were equal. This suggests a specialised method for quantitative assessment of the drug in the commercial formulation.

In an acidic buffer, BRH and the dyes BTB, BPB, and BCG create ion-pair complexes. These complexes are extracted quantitatively into chloroform. The maximum absorption was at 415 nm for the ion-pair complexes containing BTB, BPB, and BCG. Similar circumstances did not consider in any absorption of the reagent blank. While the aromatic amine group in BRH is protonated in an acidic environment, the sulphonic acid group in BTB is the sole one that undergoes dissociation in the PH range of 1 to 5. Sulphonepthalein dyes include BPB and BCG as examples. Such dyes' colour outcomes from the opening of the lactoid ring and subsequent production of the quinoid group. The two tautomers are thought to be present in equilibrium, but only because of how strongly acidic the sulphonic acid group is.

Discussion

When kept in the refrigerator, the prepared stock and working solutions were stable for up to 21 days and did not consider in the production of degraded compounds when used in experiments. The peak purity during the validation studies was 0.985 or higher. On alkaline stress with retention time, gemcitabine generates six distinct breakdown products. Two degradation products of gemcitabine have been reported, according to the percentage of remaining gemcitabine. The chromatogram, contour plot, peak purity, and UV spectra of gemcitabine and its degradation products. The collected UV spectra show that gemcitabine or its intermediates are the source of all of the breakdown products. The presence of a degraded product that is coeluted with gemcitabine but does not absorb UV light at 275 nm. The suggested methods have been verified using the ICH recommendations for selectivity, specificity, accuracy, and precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness, and regression equation. A technique has been used as a comparison point for the Student's test and variance test. Five replicate analyses of 15.0 g mL of BRH were performed in

order to assess the suggested methodologies' repeatability. All of the processes had a coefficient of variation that was less than 1.2%. The determination of BRH in pharmaceutical formulations has been accomplished using the suggested methodologies. The outcomes, which are displayed in contrasted with those from a technique using a -test with a 95% level of confidence.

Conclusion

For the purpose of detecting gemcitabine in formulations, a validated HPLC method has been created. The suggested stability signalling approach is easy, affordable, exact, particular, accurate, and sturdy. The method can separate various drug degradation products that can be calculated independently. The resilience of chromatographic separation was tested using the experimental design, which proved to be very helpful throughout the validation stage [10]. The routine analysis of gemcitabine in pharmaceutical dose form can therefore be carried out using this technology simply and conveniently. The suggested spectrophotometric methods offer easy, accurate, and reasonably priced analytical procedures for the determination of BRH in pure or tablet dosage forms without the interference of usual excipients. In addition, the new procedures save time and do not need the complex pre-treatments associated with chromatographic methods. They can be used for routine analysis in quality control laboratories thanks to these qualities.

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Conflicts of Interest

The author has no known conflicts of interested associated with this paper.

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