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Development and Validation of Stability Indicating HPLC Isocratic Method of Dota and Family Compounds for its Qualitative and Quantitative Analysis in Bulk Drug Use in Green Mobile Phase

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Abstract:

The previously described gradient elution and complex mobile phase HPLC techniques for determining gemcitabine are time-consuming. The goal of this work is to create an efficient HPLC technique for the simultaneous determination of DOTA and associated compounds utilized as intermediates in the manufacture of API. as part of an API, researching the manufacture of DOTA and related compounds at various stages (such as CYCLEN, DODIA, and DOTRA) These findings show the usefulness and potency of this straightforward isocratic HPLC technique in assessing the general performance of a formulation containing gemcitabine.

Keywords: Development, Stability, Hplc, Dota, Drug and Mobile Phase

Introduction

Potentially useful compounds might be uncovered by the systematic testing of hundreds or thousands of molecules. When we understand the molecular pathophysiology of illness, we may use this information to rationally develop new drugs or improve upon those already on the market. The effects and toxicity of promising substances are examined in animals early in development. Compounds that show promise in animal investigations may go on to human trials Exemption permits for the use of investigational novel drugs (INDs) are given out by the FDA when a protocol outlining the clinical trial has been authorized by the IRB and the FDA. A compound's patent term starts at this moment, often granting the owner twenty years of monopoly, but the drug won't be available for sale until the FDA greenlights it.

Everyone has the right to take care of their health and flourish. Article 48 of the Indian Constitution mandates that the government make health care a top priority. Health care improvement efforts at the federal and state levels have been aided by the Five-Year Plans. Significant progress has been made since independence in improving the health status of the people, as evidenced by the eradication or control of diseases like smallpox, malaria, etc., the reduction of mortality rate, the increase in life expectancy, the establishment of a fairly extensive network of health care institutions,

and the availability of a large stock of medical and health personnel.

To improve public health throughout the nation, the National Health Policy of 1983 was a watershed event. India has reaffirmed its commitment to the Millennium Development Goal of "Health for all by the year 2000 A.D." by ensuring that all of its residents have access to high-quality basic care. To get there, we need to speed up the production of all health care inputs, including high-quality pharmaceuticals and vaccinations that save lives. The provision of healthcare cannot rely just on pharmaceuticals. However, they serve a vital purpose in preserving, maintaining, and restoring public health, not to mention population control, when handled sensibly. As a result, when it comes to providing the basic health care needs of the Indian people, the pharmaceutical industry plays a pivotal role.

LITERATURE REVIEW

Swamy, M et.al (2023). According to this article, bio-analytical techniques are widely used for quantifying pharmaceuticals and their intermediate products in tube matrices, and these techniques need to be amenable to acquisition in human clinical and research settings. In computing and rendering BE, PK, and TK survey, the bio-analytical technology used for quantitative estimate of medications and their metabolites in natural media plays an important part. System enhancement, system confirmation, and statistical distribution research are at the forefront of the BA process. For the BA of drugs in the body, techniques like as HPLC and LC conjugated LCMS-MS are used.

Taylor, David. (2016). The manufacturing, use, and disposal of medications all pose potential environmental risks owing to the release of residues into the environment. Medicines, however, play an essential role in both the treatment and prevention of disease in humans and other animals. Recent research has examined this exposure's potential environmental impacts. This book explores the processes involved in creating and disposing of pharmaceutical residues, as well as their degradation mechanisms and subsequent impacts on wildlife and human health. This book covers the existing situation and discusses potential probable implications, making it necessary reading for pharmaceutical industry researchers, environmentalists, policy makers, and students enrolled in courses in pharmacy and environmental science.

Campanelli, Matteo. (2020). This chapter primarily focuses on broad strategies and concerns for developing chromatographic techniques for separation, identification, and quantification of pharmaceutical substances that may be used throughout the spectrum of drug development tasks. In addition, the challenges and factors that should be taken into account while validating analytical techniques are discussed in this chapter.

Chaudhary, Avinash. (2023). The pharmaceutical sector now relies heavily on HPLC-CAD (high-performance liquid chromatography with charged aerosol detection). This article's goal is to provide a summary of HPLC-CAD's applications in the field of pharmacometrics. The advantages of high-performance liquid chromatography-mass spectrometry (HPLC-CAD) over other detection technologies will be discussed, as will its applications in drug quantification, impurity analysis, stability studies, and bioavailability investigations. Future prospects for the subject are reviewed, and the benefits and drawbacks of HPLC-CAD are analyzed.

Tiwari, Gaurav et.al (2023). For submission of a registration application in the European Union (EU), Japan, and the United States, the ICH Q1A guideline details what must be included in the stability

data package for a new drug substance or drug product. The scope does not include export testing or international certification. The document is meant to serve as an example of the stability data package required for novel therapeutic substances and products, but it leaves plenty of room for a wide range of possible practical situations that may arise due to specific scientific considerations and properties of the materials being evaluated. It is possible to use different approaches if there are scientifically sound justifications for doing so.

RESEARCH METHODOLOGY

Instrumentation and software:

To provide a high-performance liquid chromatographic method for the qualitative (%Purity) and quantitative (%Assay) analysis of DOTA and related (family) compounds simultaneously. To achieve chromatographic separation, we used a Supelco Astec CyclobondTM I 2000 RSP (250 4.6 mm 5 m) and a Phenomenex HPLC synergi polar RP (250 4.6 mm 4 m) column for the analysis. The quaternary pump, microcontroller, and variable wavelength detector (VWD) from Agilent's HPLC 1100 series were employed. In this study, we used a 0.45 m membrane filter, an analytical balance, and a digital pH meter.

Chemicals and reagents:

DOTA and its three related chemicals, all of which are pharmaceutical grade, are 99.9% pure. The HPLC-grade milli-Q water and orthophosphoric acid (H3PO4) used in this work were both purchased from a reputable supplier.

Details of method:

Chromatographic conditions:

Sr. No.	Parameter		Condition for Qualitative (%Purity)	Condition for Quantitative (%Assay)
1.	Preparation of buffer	:	Weighted and transferred accurately 2.8gm of KH_2PO_4 (Potassium dihydrogen orthophosphate) and add 0.8mL of orthophosphoric acid into a beaker containing 1000mL of water and adjusted pH 2.40±0.10 with orthophosphoric acid(15% v/v.) mixed it well. Filtered it through 0.45µm membrane filter and sonicated to degassed.	Transferred 0.8mL of Orthophosphoric acid into a beaker containing 1000mL of water mixed it well. Filtered it through 0.45µm membrane filter and sonicated to degassed.
2.	Preparation of mobile phase	:	Used degassed of buffer.	Used degassed of buffer.
3.	Diluent	:	Used water as diluent.	Used water as diluent.
4.	Column	:	Supelco Astec CyclobondTM I 2000 RSP (250×4.6mm, 5µm)	Synergi Polar RP, (250×4.6mm, 4µm)
5.	Column oven temperature	:	35°C	35°C
6.	Detection	:	195nm	200nm
7.	Injection volume	:	10.0µL	5.0µL
8.	Run time	:	20.0 min.	20.0 min.
9.	Flow rate	:	0.8mL/min.	1.0mL/min.
10.	Pump mode	:	Isocratic	Isocratic

Solution making preparations:

Solution for system compatibility and validation of retention time with high specificity:

DOTA, CYCLEN, DODIA, and DOTRA were each weighed and deposited into a 50 mL volumetric flask; 25 mL of diluent was added, sonicated to dissolve the drug, and the flask was filled to the mark with more diluent. The concentration of DOTA, CYCLEN, DODIA, and DOTRA in the solution was 200g/mL (200ppm).

Test solution:

Accurately weighed and pipetted a 5mg sample of DOTA into a 25mL volumetric flask, followed by adding 10mL of diluent to each flask, sonicating to dissolve the contents, and then filling the flasks to the mark with more diluent. The concentration of DOTA in this solution is 200 ppm (or 200 g/mL).

Standard stock solution:

We measured out 250 mg of DOTA, CYCLEN, DODIA, and DOTRA into four separate 50 mL volumetric flask, dissolved the substances with sonication, adjusted the concentrations to the proper levels and ensured a smooth blend. They utilized a DOTA, CYCLEN, DODIA, and DOTRA solution at a concentration of 5000 g/mL (or 5000 ppm).

DATA ANALYSIS

Improvements to chromatographic settings:

Green chemistry without solvent simply the aqueous buffer mobile phase has been accomplished thanks to the development of quantitative (%Assay) and qualitative (%Purity) HPLC procedures.

Due to its importance in quality control, analytical research has focused extensively on improving quantitative (%Assay) HPLC procedures for determining pharmaceuticals. This method is novel while yet being adaptable, generalizable, and fundamental. It's user-friendly, so scientists put it to good use. Method development aims mostly at locating the DOTA. UV detection well area is accomplished using Phenomenex HPLC synergi Polar RP, column 80A, (2504.6mm, 4m) or comparable due to the other components that required an almost exclusively water mobile phase. One thousand milliliters of water and eight milliliters of orthophosphoric acid were combined in a beaker to make mobile phase. After sonicating it to get rid of the air, we filtered it using a 0.45 m membrane. The elution operation, including column re-equilibration, takes around 10 minutes at a mobile phase flow rate of 1.0 mL/min. Use water as the diluent, set the UV detection wavelength at 200 nm, inject 5 L, and heat the column oven to 35 C.

Table-1: System suitability result

Sr.No.	Figure no.	Compound name	Assay of area	Purity of area
1	3 and 4	DOTA	477325	280015
2	5	DODIA	-	278652
3	6	DOTRA	-	272508
4	7	CYCLEN	-	253056

Method validation:

We used the ICH recommendations for "Validation of analytical procedures: text and Methodology" to validate our technique for determining DOTA. System and method suitability were shown by demonstrating the following: Q2(R1)" Q2A and Q2B with standards bulk drug; precision (reproducibility), intermediate precision, accuracy, limits of detection and quantification, short-term and long-term stability of the analysts in the prepared solutions, selectivity, specificity, linearity, range, and precision (reproducibility). Stability. **System suitability:**

The findings of the system suitability test are crucial to establishing the validity of the analytical method. In this investigation, we used a system suitability solution composed of DOTA and related intermediate chemicals. With constant values for DOTA retention time (tR), peak area (A), relative retention times (RtR), and resolution (RS) between nearby peaks, the example curves offer a good separation numerically..

Linearity and range on LC-UV:

Linearity study:

Standard stock solution for linearity:

By placing 25 mg of DOTA standard into a 50 mL volumetric flask, adding 5 mL of each sample, and finally adding 10 mL of diluent and sonicating to dissolve in an ultrasonic water bath, we were able to prepare concentrations of DOTA (500g/mL, 500ppm), CYCLEN (500g/mL, 500ppm), and DODIA (500g/mL, 500ppm).

To conduct a DOTA test, a series of substances are injected into a chromatographic system with a linearity range of 150% (300 g/mL) to 50% (100 g/mL). Keep the chromatogram in mind. Keep tabs on the observable area.

To ensure that your CYCLEN, DODIA, and DOTRA compounds are as pure as possible, use this procedure to inject them sequentially into a chromatographic system with a linearity level between 150% (300 g/mL) and 0.5% (1 g/mL). Think about the chromatogram. Keep track of the analyzable region. Determine the test method's Correlation Coefficient and Y-intercept by calculating the Area vs. concentration.

The coefficient of determination (R2) was more than 0.995 across all calibration curves for DOTA, CYCLEN, DODIA, and DOTRA. Each calibration curve and result was put through a test for goodness-of-fit.The memory findings shown in Tables 3.2–6.6 and the chromatographic results all have R2 values that indicate that the system is suitable. There would be no less than a 0.98 correlation

Table 2: Linearity and range for DOTA.

Sr.no.	Linearity % level	µg/mL	Assay of Area (mAU)	Purity of Area(mAU)
1	0.5	1	-	1350
2	1	2	-	2762
3	2.5	5	-	6810
4	5	10	-	13560
5	10	20	-	27590
6	25	50	-	70152
7	40	80	-	110725
8	50	100	241025	137305
9	80	160	382001	221290
10	100	200	477255	280015
11	120	240	576391	338255
12	150	300	716254	411502
Correlation co-efficient			0.999	0.999
Slope			4767.11	2759.46
Intercept			1873.99	41.82

Table-3: Linearity and range for DODIA.

Sr.no.	Linearity % level	µg/mL	Area(mAU)
1	0.5	1	1380
2	1	2	2790
3	2.5	5	6890
4	5	10	13856
5	10	20	27825
6	25	50	68825
7	40	80	110520
8	50	100	139258
9	80	160	220562
10	100	200	278652
11	120	240	329952
12	150	300	412525
Correlation co-efficient			0.999
Slope			2775.56
Intercept			291.30

Table-4: Linearity and range for DOTRA.

Sr.no.	Linearity % level	µg/mL	Area(mAU)
1	0.5	1	1350
2	1	2	2758
3	2.5	5	6825
4	5	10	13825
5	10	20	27125
6	25	50	67825
7	40	80	108256
8	50	100	142582
9	80	160	216258
10	100	200	272508
11	120	240	339870
12	150	300	404259
Correlation co-efficient			0.999
Slope			2797.24
Intercept			307.29

Table-5: Extent and linearity of CYCLEN.

Sr.no.	Linearity % level	µg/mL	Area (mAU)
1	0.5	1	1280
2	1	2	2590
3	2.5	5	6410
4	5	10	13250
5	10	20	25391
6	25	50	63528
7	40	80	101180
8	50	100	126552
9	80	160	214501
10	100	200	253056
11	120	240	303005
12	150	300	380025
Correlation co-efficient			0.999
Slope			2527.51
Intercept			532.43

Table-6: Synopsis of Linearity Test for Suitability to System, R^2, t_R, Rt_R, Y-intercept, LOQ and LOQ Data.

Compound	Range (µg/mL)	t_R	About Rt_R,	LOQ (µg/mL)	LOD (µg/mL)
DOTA(Purity)	1 to 300	7 to7.5	1	4.14	1.36
DOTA (Assay)	100 to 300	7 to 7.5	1	-	-
CYCLEN	1 to 300	4.2 to 4.5	0.60	1.68	0.54
DODIA	1 to 300	6.5 to 7	0.91	2.56	0.84
DOTRA	1 to300	5.5 to 6	0.78	14	4.66

$t_{R=}$ Retention time, R^2= Coefficient of determination, $Rt_{R=}$ Relative retention time.

CONCLUSION

An innovative to determine Aniracetam and its three related impurities, 4-Methoxy benzoic acid, N-anisoyl GABA, and 2-pyrrolidinone, simultaneously in the bulk drug, synthesis drug, drug intermediate, and tablet formulation, a reversed-phase high performance liquid chromatography method was developed, validated, and shown to be sensitive, accurate, precise, and robus the isocratic mobile phase employed in the established HPLC analytical technique was aqueous buffer. This method was developed and validated in accordance with ICH recommendations for the rapid, accurate, easy, and selective identification of DOTA and DOTA family compounds during intermediate synthesis of bulk medicines, making it useful for both qualitative (purity by area) and quantitative (assay w/w) analysis of the sample.

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