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A STUDY OF R-LACOSAMIDE USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

CANDIDATE NAME = JADHAV VIJAY BHAUSAHEB
DESIGNATION- RESEARCH SCHOLAR MONAD UNIVERSITY HAPUR U.P
GUIDE NAME = DR. T. VINAY KUMAR
DESIGNATION= (ASSOCIATE PROFESSOR) MONAD UNIVERSITY HAPUR U.P

ABSTRACT
Accurately quantifying R-Lacosamide (R-LCM) in the presence of S-Lacosamide (S-LCM) in a variety of circumstances, especially pharmaceutical formulations and biological samples, necessitated the present work. The R-enantiomer of lacosamide, an antiepileptic medication, is responsible for the drug's therapeutic effectiveness. It is crucial to precisely discriminate between and quantify the two enantiomers because it is co-administered with its S-enantiomer. There are several reasons why this distinction is crucial. To begin, R-LCM and S-LCM are very different in terms of their pharmacological effects. While S-LCM is deemed pharmacologically inactive, R-LCM has the necessary antiepileptic action. Therefore, it is essential for therapeutic efficacy that R-LCM be present in pharmaceutical formulations in sufficient quantities. On the other hand, an overabundance of S-LCM could increase production costs for pharmaceutical companies and expose patients to unwarranted risks. As a second point, pharmacokinetic research relies heavily on disentangling the effects of R-LCM and S-LCM on drug metabolism, distribution, and elimination. This information is priceless for adjusting dose schedules, reducing the risk of side effects, and personalizing care for each patient.

KEYWORDS: R-Lacosamide, Liquid Chromatography, Mass Spectrometry, pharmaceutical formulations

INTRODUCTION

Analytical chemistry is both the science and art of using analytical processes to determine material properties such as composition, purity, safety, and quality. The goal is to advance the theory behind and scientifically elaborate on existing analytical methods, as well as to refine and provide empirical support for those approaches. Nutritional value assessment, monitoring environmental quality, and biopharmaceutical and bioequivalence investigations are just some of the many areas where this technique has been put to use.

The United States Food and Drug Administration and other governing agencies oversee the pharmaceutical business. To maintain the integrity of products on the market, they want research into process related impurities (IMPs) and degradation products (DPs) formed in the drug ingredients and formulation.

1-8 Any impurity that isn't intended to be in the drug formulation dilutes the effectiveness of the drug. In addition to byproducts and DPs, impurities can also take the form of interaction products, intermediates, and related products.9 There are a number of potential impurity sources in the drug manufacturing process.

Both the drug ingredient and the inert material used in drug product formulation might contribute to impurities in the drug. Excipients may be the source of these problems.

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Excipients with contaminants may cause chemical instability. Organic or inorganic substances, volatile or nonvolatile, inactive or with greater pharmacological activity: all these and more might be considered impurities.

However, assessment is required to assert suitable content and, by extension, dose. By understanding how degradation byproducts are created, deterioration can be mitigated or avoided altogether.

Drug product impurity management is important for a number of reasons, including patient health and safety, revenue and ethics, and market share. Analytical approach for impurity investigation plan is depicted in Fig. 1.

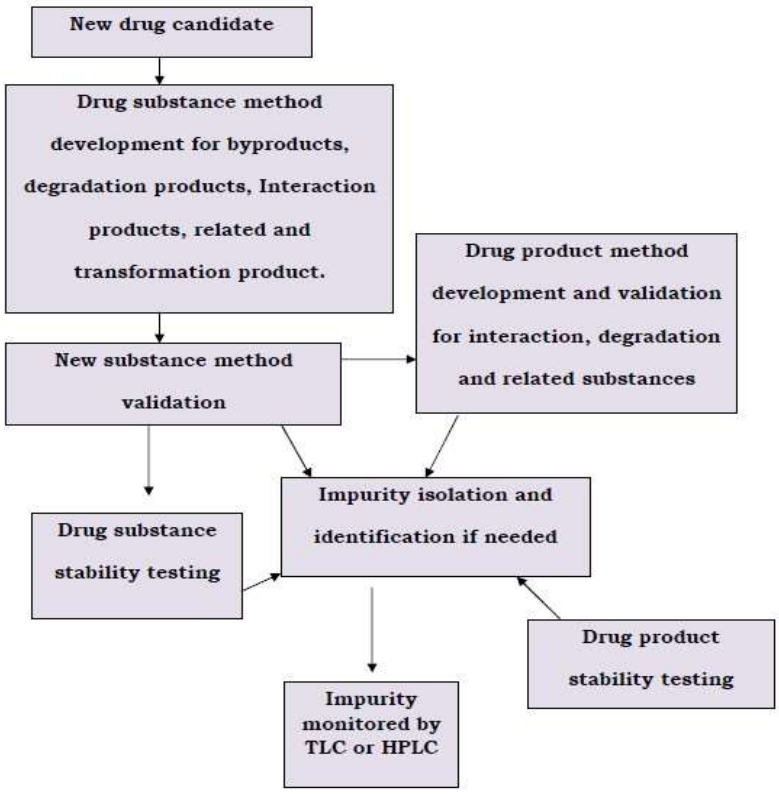

Figure 1 Overall strategy in use of analytical method for investigation of impurities.

Stress testing can produce DPs in a shorter amount of time than traditional stability investigations. The ideal stability indicating analytical technique (SIAM) would separate the parent medication from all its degradation products and identify them quantitatively.10 The creation of a stability signaling method relies heavily on stress testing. The medication molecule's intrinsic stability can also be evaluated. Speculating on what might cause the drug's active ingredient to break down is helpful. Accelerated temperature (thermolytic), severe pH (acidic, basic, neutral hydrolysis), and oxidative and photolytic conditions are all part of this sort of investigation, which is conducted in accordance with ICH recommendations and intended to ensure the safety and efficacy of the medicine. The optimum formulation can be developed with a deeper knowledge of the drug's degradation behavior and its interaction with the excipients.

The impurities listed below can be generated using the following reactive species.

1. Water (can hydrolyze some drugs)
2. Small electrophiles (aldehydes, carboxylic acid derivatives)

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3. Peroxides (Can oxidize some drugs)
4. Metals (Can catalyze oxidation and other drug degradation pathways)

Water might come from excipients, pharmacological ingredients, or the ambient during manufacturing. The rate of hydrolysis is dependent on the water activity of the drug's surface. Normal functional groups in pharmaceutical substances are more susceptible to hydrolysis by acids or bases. Aldehydes, reducing sugars, and metal ions can all react with the amino acids released when gelatin is hydrolyzed. Drug hydrate formation in water can lead to decreased crystallinity, increased chemical instability and different dissolving kinetics. Hydrolysis of excipients may be hastened.

UV-VISIBLE SPECTROPHOTOMETRY:

Beer-Lambert's law, as described below, forms the basis for quantitative UV measurements.

A = constant*bc

Where A= absorbance b= pathlength in cms. Constant may be a, ε or $A^{1\%}_{1\ cm}$ a = absorptivity when concentration of analyte is in gms/lit. ε = molar absorptivity when concentration of analyte is in moles/lit.

$A^{1\%}_{1cm}$ = absorptivity when concentration is expressed in gms/100 ml.

It can be applied to multicomponent analysis to a limited extent.

Absorbance of the component must be additive in order to do the analysis. Beer's law must be satisfied by all components at all measured wavelengths. The following procedures are commonly employed.

1) Simultaneous equation/ Vierordts method:

This technique works well when the two substances have sufficiently different maximum absorption wavelengths and do not react chemically with one another. Prior to any development, it is essential to verify the absorbances' additive nature. Take the absorbance spectra of Y and Z in Fig. 1 as examples of a multicomponent system. 1.2, λ_1 and λ_2 being the wavelength of maximum absorbance (λ_{max}) of Y and Z respectively. A_2 and A_1 are absorbances of the test solution at λ_2 and λ_1 respectively and c_Y and c_Z are the concentrations of Y and Z in the sample solution respectively.

The absorptivities of component Y at λ_2 and λ_1 are a_{Y2} and a_{Y1} respectively. The absorptivities of component Z at λ_2 and λ_1 are a_{Z2} and a_{Z1} respectively.

Then the absorbances of the sample mixture at λ_1 and λ_2 may be expressed by forming two simultaneous equations -

$$A_1 = a_{Y1}bc_Y + a_{Z1}bc_Z \quad \text{at } \lambda_1 \text{ …….. (1)}$$

$$A2 = aY2bcY + aZ2bcZ \quad \text{at } \lambda2 \text{ …….. (2)}$$

Using equations (1) and (2), the quantitation of Y and Z in the test mixture can be performed.

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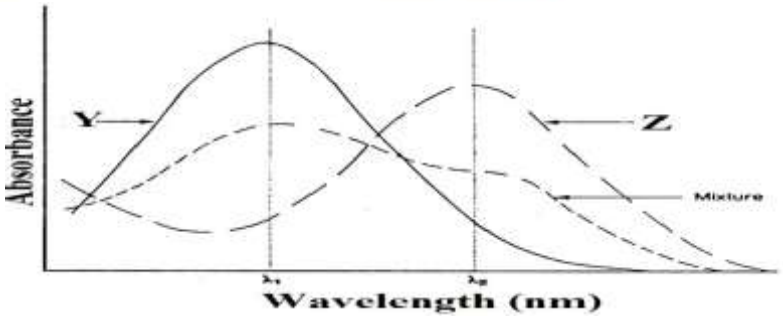

Figure 2 Overlay spectra of substance Y and Z

2) Q-analysis/ Absorbance ratio method:

Hufner's quotient (or Q value) is calculated as part of this approach. The absorbances are determined at the isoabsorptive point in the quantitative assay(λ_1) and at λ_{max} of one of the component (λ_2) Component Y and Z concentrations, CY and CZ, are presented in Fig. 1.3 and can be calculated using Eqs. 5 and 6.

$$cY = (QM - QZ/ QY - QZ) \times A1/ aY1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

$$cZ = (QM - QY/ QZ - QY) \times A2/ aZ1 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(6)$$

$Q_M = aZ_2 / A_1$, $QZ = aY_2 / aZ_1$, $QY = A_2 / aY_1$

A_2 and A_1 are the absorbances of admixture at λ_2 and λ_1 respectively.

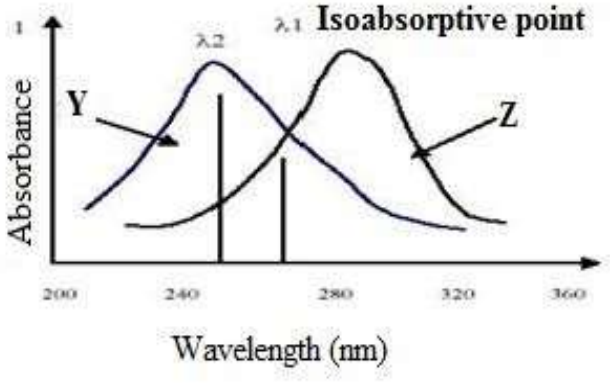

Figure 3 Overlain spectra of substance Y and Z

3) Derivative Spectrophotometry:

As can be seen in Fig. 4, this process entails transforming zero-order spectra into higher-order ones. It is possible to quantify the spectrum's slope as a function of wavelength.

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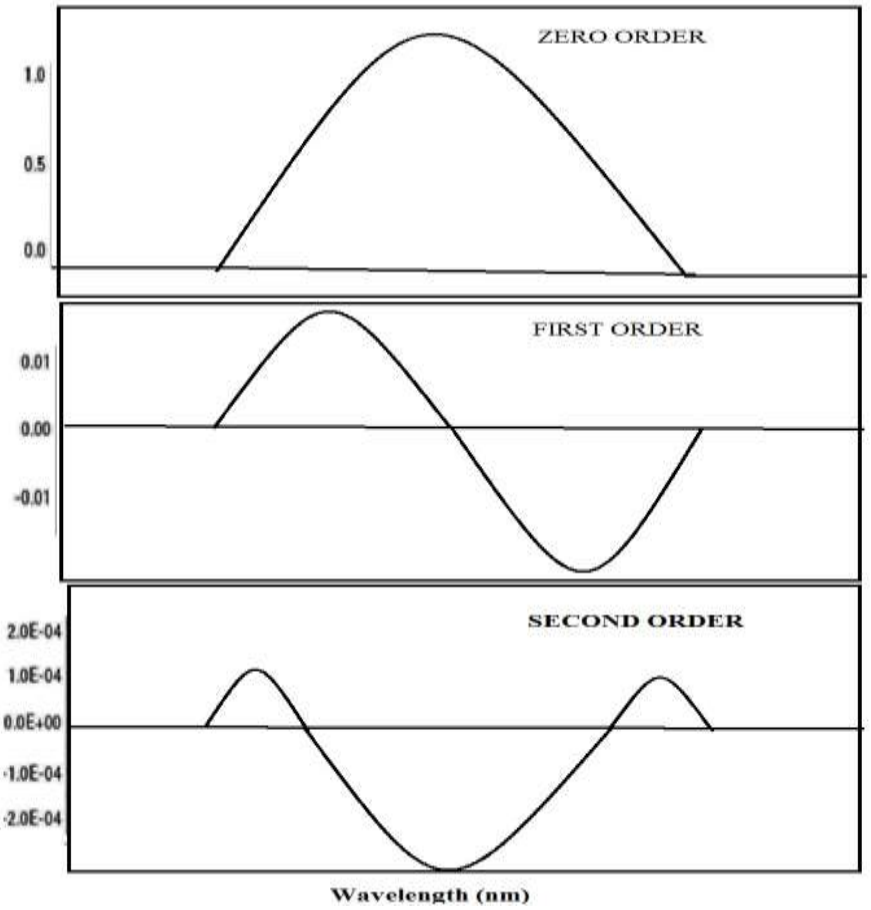

Figure 4 Absorbance and derivative spectra of a gaussian band

HPLC

Due to the smaller particle size of the solid support that is coated with a thin liquid layer as a stationary phase (SP), high-performance liquid chromatography (HPLC) has evolved as an enhanced form of liquid chromatography. This is because there is very little room for diffusion in the SP, so the equilibrium between the phases is reached very quickly. To separate the liquid from the solid, a mobile phase (MP) is pushed through the column at high pressure, and the liquid is chemically bound to the surface of the silica solid support.19 Many free hydroxyl groups on the surface of silica gel can be converted into a substituted silylating agent, such as octadecyldimethylsilyl chloride, by a chemical process. Unreacted hydroxyl groups can be treated with trimethyl silyl chloride through a capping method to reduce adsorptive effects.

Particle-based (silica and polymer-based) SPs, monolithic (silica and polymer-based) SPs, SPs with hydrophilic interaction (HILIC), SPs with mixed beds, hybrid packing, and SPs based on zirconia or titania are only few of the many varieties of SPs in use.20 Fully porous packings offer large specific surface area (200-300 m^2/g), larger retention and loadability. When injected at higher rates, these are less likely to show large peaks. Analyte access should be determined by pore size. Small molecules (mol.wt-100-500) need pore size 10 nm. Larger molecules require a pore size of 30 nm to be retained effectively. The flexibility to control surface area and pore size, as well as its high mechanical strength and the availability of known surface modification techniques, make silica an attractive material. When deciding on an MP, it's important to take into account the solvents' viscosity, compressibility, refractive

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index, UV cut-off, polarity, vapor pressure, and flash point. Table 1 lists the various ways in which partition chromatography can be categorized.

Table 1 Classification of partition chromatography

No.	Type	Characteristics
1.	Normal phase chromatography	SP- Polar, MP-Nonpolar It prefers retention of polar compounds and elution of nonpolar compounds.
2.	Reversed Phase Chromatography	SP- Nonpolar (Water, Acetonitrile, Methanol), MP- Polar Nonpolar solutes are retained favouring elution of polar solutes
3.	Ion Supression Chromatography	Useful for compounds like weak acid (pKa >2) and weak bases (pKa < 8). These are ionized partially at neutral pH values characteristic of usual MP. At pH below dissociation constant, free acid predominates and partitions into SP as it is less polar than anion. At pH above the dissociation constant, weak acid exist in the anionic form.
4.	Ion Pair Chromatography (Ion pair reagents: Heptanes sulphonic	Useful for strong acids or bases that are ionized throughout the pH range (2-7). Reagents which dissociate to produce ions opposite in charge to that of solute
	acid for cationic species and tetra-nbutlyammonium hydroxide for anionic substances)	is added. Added ion may react directly to the charged solutes forming nonpolar ion pair which partitions in SP or nonpolar end of ion pair agent partition into SP leaving its polar end out to behave as ion exchanger for separation.
5.	Soap Chromatography	It is a type of ion pair chromatography which uses detergent or soap.
6.	Metal ion complexation	Olefinic compounds are separated by adding of small amount of metal ion.
7.	Centrifugal partition chromatography	It is a combination of countercurrent and partition chromatography.

The most consistent samples should be converted to reversed phase. The polarity of the MP can be altered by adding or removing solvents such as ethanol, methanol, acetonitrile, dioxane, tetrahydrofuran, and dimethylformamide from water.

Nonpolar normal-phase MPs, such as hydrocarbons and ethers, are potentially flammable. In the isocratic elution mode, the composition of the mobile phase does not vary during the separation process.

CONCLUSION

Pharmaceutical formulations containing both individual drugs and combinations of drugs have had stability indicating analytical procedures developed and verified. Ketorolac tromethamine, tramadol hydrochloride, dapoxetine hydrochloride, sildenafil citrate, hydrochlorothiazide, and telmisartan can all be analyzed simultaneously using the established methodologies, and the procedures can be used on a regular basis. The study yielded useful data on the degradation patterns of the medications. The capsule formulation of ketorolac tromethamine and tramadol hydrochloride in distilled water can be easily measured using current UV techniques. Absorbance at 246 nm and 271 nm was measured as part of the simultaneous equation method used to analyze the mixture. nm, λmax of KTT and TMH respectively. The method was found to be linear in the concentration range of 2-60 µg mL^{-1} and 5-150 µg mL^{-1} respectively for KTT and TMH. Hydrochlorothiazide and telmisartan in a commercial tablet formulation were quantified using a stability-indicating HPTLC technique.

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