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A STUDY OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC) TOWARDS HIGH PERFORMANCE THIN LAYER

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ABSTRACT
The ability of an analytical method to measure R-LCM while also taking into account the presence of S-LCM is also crucial in the field of bioanalysis, which involves quantifying medication concentrations in biological samples. The development of new medicines or the evaluation of treatment outcomes can be jeopardized by inaccurate quantitative results from clinical trials. A reliable Liquid Chromatography-Mass Spectrometry (LC-MS) approach for the quantification of R-Lacosamide in the presence of S-Lacosamide is needed to solve these crucial difficulties. Improved knowledge of the pharmacokinetics and bioavailability of lacosamide will result from the application of such a technique, which will also aid in the quality control of lacosamide-containing medications. The ultimate goals of this study are to better care for patients, manage epilepsy and other neurological illnesses, and guarantee the safety and effectiveness of lacosamide-based medicines.

KEYWORDS: Ultra Performance, Liquid Chromatography, Thin Layer, pharmacokinetics, bioavailability, lacosamide-based medicines.

INTRODUCTION

The analytical procedures developed in-house by the pharmaceutical companies presenting these formulations are rarely shared with the scientific community. Therefore, researchers examining these medicines may lack access to appropriate analytical tools. Therefore, only medications and combinations for which no analytical methods are available in the literature or for which minimal work is undertaken were selected for this study. Impurities and degradation products formed during stress testing have also been the subject of the effort.

Different analytical methods are taken into account in this work to create SIAM for single drug or combination pharmaceutical formulation, including HPLC, UPLC coupled with UV, PDA and mass detector, and HPTLC. Degradation products may have their structures investigated using high-resolution mass spectrometry. The use of UV-visible spectroscopy is easy, quick, practical, and cheap. Postoperative pain and inflammation are commonly treated with a combination of tramadol hydrochloride and ketorolac tromethamine. The assay of this combination formulation cannot be found in the literature. Therefore, it was thought to be beneficial to create UV technologies that can simultaneously quantify these medications and be used in lab settings.

UV approaches can be useful in specific situations, but they are challenging to design in a stability indicating environment. This is because medications and their breakdown products share comparable UV spectra, as a result of their shared chromophore. The correct wavelength for a measurement is difficult to determine when there is whole or partial overlap

between UV spectra. The pharmaceutical industry and authoritative encyclopedias both generally embrace HPLC techniques. Because of this, it was determined to create an HPLC method for analyzing racecadotril in capsule form. Furthermore, there has been no research done on the subject of determining which medication degradation products actually exist. So, mass spectrometry was chosen to suggest potential breakdown products. Ofloxacin and ketorolac tromethamine need an approach to estimation from an eye drop formulation as well. UPLC is a modern quantitative analysis technology that developed from HPLC. Rapid, specific, and sensitive SIAM for quantification of a commercial tablet formulation of sildenafil citrate and dapoxetine hydrochloride were additional goals of this investigation. Since the fragmentation pattern and degradation products for this mixture have not been described, LC-MS/MS and LC-MS were chosen for this investigation.

The use of HPTLC in the regulation of herbal product testing. It requires less time and money to prepare samples for examination. It was thought to be the best technique for measuring rutin concentrations in Carica papaya leaf tablets. Due to the sample's complexity, high resolution mass spectrometry was employed to identify any phytoconstituents present in the finished product. SIAM development using HPTLC for telmisartan and hydrochloride in combination tablet determination is also under consideration.

To ascertain the presence or absence of medicines or metabolites in body fluids, bioanalytical techniques are employed. During pre-clinical and clinical pharmacology investigations, they are playing a crucial role in determining bioequivalence, pharmacokinetics, and toxicokinetics. MS-MS systems are superior to HPLC and UV detection in many situations, including the analysis of complex mixtures that often require little in the way of sample pretreatment. In addition, when dealing with complex mixtures, the HPLC assay may not be as sensitive or specific as desired. Some metabolites formed from a heavily metabolized molecule may share the original compound's retention period and UV spectral properties. As a result, MS-MS became a viable option for bioanalysis.

Selected Reaction Monitoring (SRM) and Multiple Reaction Monitoring (MRM) both keep tabs on a certain fragment ion that can be measured in a large data set. Typically, there is only one peak on an SRM plot. To identify R-Lacosamide in human plasma, a direct chiral tandem mass spectrometric technique was created.

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

Higher mobile phase flow rates and smaller particle sizes in the stationary phase are hallmarks of UPLC. This provides benefits such as enhanced speed, sensitivity, and resolution. It lessens the amount of solvent used. The van Deemter equation states that as particle size decreases, ($\leq$ 2.5 μm), Gains in efficiency are substantial, but the back pressure rises quickly. The number of theoretical plates (N) in a separation process directly correlates to the resolution of the process. Particle size is inversely proportional to N. When particles are shrunk from 5 m to 1.7 m (a factor of three), the number of particles (N) increases by three and the number of pixels (resolution) increases by the square root of three (1.7).27, 28 The effectiveness is proportional to the column length and inversely to the particle size. Therefore, the resolution is maintained despite a decrease in column length of the same order as the particle size. Using UPLC allows for better separation thanks to the identification of more peaks and an increase in the overall

quality of the spectra. The high efficiency, non-porous particles (1.5 µm) Gains in efficiency are substantial, but the back pressure rises quickly.26 The number of theoretical plates (N) in a separation process directly correlates to the resolution of the process. Particle size is inversely proportional to N. When particles are shrunk from 5 m to 1.7 m (a factor of three), the number of particles (N) increases by three and the number of pixels (resolution) increases by the square root of three (1.7). The effectiveness is proportional to the column length and inversely to the particle size. Therefore, the resolution is maintained despite a decrease in column length of the same order as the particle size. Using UPLC allows for better separation thanks to the identification of more peaks and an increase in the overall quality of the spectra.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC):

It has high separation power, high performance, and high reproducibility, making it a potent analytical tool. HPTLC uses a thinner sorbent layer of only 100 m instead of the standard 250 m used in TLC. Rapid analysis is made possible by the small particle size that allows for separation in only 3-5 cm. Adsorption and solubility differences are the separating factors. The phases of HPTLC analysis are depicted in the following diagram (Fig. 1).

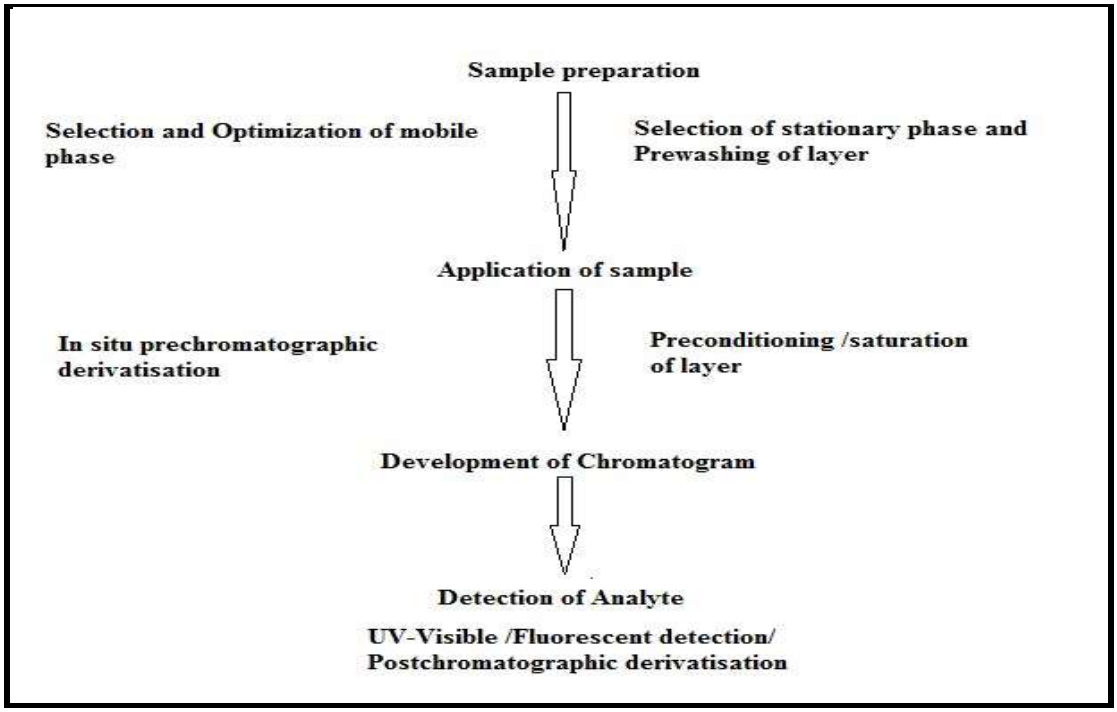

Figure 1 Steps involved in HPTLC analysis

Silica gel 60F, chemically modified silica gel, and aluminum oxide (alumina) are all examples of stationary phases. Generally plates of 20×20 cm or 5×7.5 cm having 100-250 µm adsorbent thickness are used. Due to HPTLC's limited sample volume, sample concentration is essential. Solvents used in extraction must be able to dissolve the analyte, be volatile, and have low viscosity. It needs to be a mild chromatographic solvent and wet the sorbent layer. Below 0.1 (Fig. 2) is the optimal Rf for analyte in the extraction solvent.

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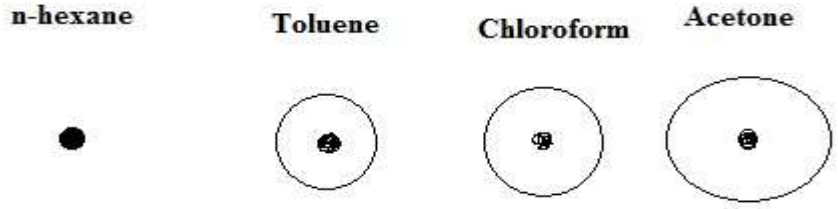

Figure 2 Chromatographic development at the spot of sample application due to change in solvent polarity.

Low-boiling-point organic solvents are the most common, and they include ethanol, methanol, acetone, and chloroform. The efficiency of sample separation is significantly affected by pre-conditioning (chamber saturation). Saturation is unnecessary for a low-polarity mobile phase, but advantageous for a high-polarity mobile phase. The composition of the mobile phase that causes phase separation should be optimized for partial saturation. Saturating the chromatography chamber with methanol or another polar solvent is necessary for reverse phase chromatography. It is via trial and error that the mobile phase most suited to the task at hand is determined, taking into account factors such as the chemical characteristics of the solute and solvent, the solubility of the analyte absorbent layer, etc. The following is an example procedure for optimizing a solvent.

a. First level: Neat solvent from different selectivity area are tested.
b. Second-level: If Rf values are too high, nonpolar solvents like n-hexane can be added to dilute the solution.
c. There are three tiers of solvent mixes possible: binary, ternary, and quaternary. Experiments used 1:1, 9:1, and 1:19 ratios. It is determined whether a tertiary mixture with a 1:1:1, 8:1:1, or 1:1:8 ratio is optimal. The pH can be adjusted by adding an acid or a base.
d. Small adjustments to the third-level proportions can be made at the fourth level. It is possible to choose the optimum mobile phase with a spot test (Fig. 3).

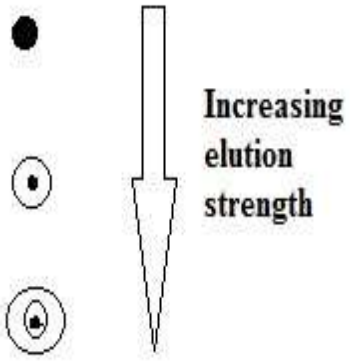

Figure 3 Spot test

Chromatographic development can take many different forms, the most frequent of which are ascent, descent, two-dimensional, anti-radial, multi-modal, forced flow planar, continuous, and radial gradient chromatography. The plates are taken out of the developing chamber and dried so that any residual mobile phase is gone.

Iodine vapor in an iodine chamber can be used for detection. Visual inspection examination at 254nm of the ultraviolet region in a UV cabinet is another method of detection. The identified spots on the computer appear as peaks after scanning the generated HPTLC plates at a wavelength chosen from the UV range. The band is transformed into peaks by the scanner, and the height or area of the peaks is proportional to the local concentration of the drug. The ratio between the compound's distance and the solvent's distance is the retention factor (Rf). The Rf values range from 0 to 1, with the optimal range being between 0.1 and 0.8.

Type of stationary phase and plate, layer thickness and binders in the layers and pH of the layer, mobile phase, solvent purity, solvent for the sample preparation, chamber size, chamber saturation, sample volume to be spotted, spot size applied, solvent level in chamber, temperature (Rf values usually increase with rise in temperature), flow rate of the solvent, velocity of the spots, and temperature all play a role in the separation and resolution of spots in HPTLC.

MASS SPECTROMETRY

In mass spectrometry, the analyte retains its charge after being ionized from the sample. Using a magnetic field, an electric field, or both in a vacuum, it can separate and identify positive and negative ions. The ions are injected to the vacuum system as a gas. Samples that are gaseous or heat-volatile can be treated in this way. Methods of desolvation or desorption are required for analytes that thermally degrade. Which ionization technique to choose in an analysis is determined by the type of analyte being analyzed and the goals of the study.33 Table 1 classifies ionization techniques as either "soft" or "hard."

Table 1. Ionization techniques used in MS

Hard ionization techniques	Soft ionization techniques
Electron Impact Chemical Ionization Field ionization	Field Desorption Fast Atom Bombardment Secondary Ion Mass Spectrometry Electrospray Ionization Atmospheric Pressure Chemical Ionization Matrix-Assisted Laser Desorption Ionization

MS-MS (Tandem Mass spectrometry)

When an analyte consists of many components, this method is employed. The first mass spectrometer uses a more gentle approach to ionization, converting different components of a mixture into corresponding molecular ions, once an analyte has been injected. A second mass

spectrometer receives these molecule ions in a sequential fashion, fragments them, and analyzes the results. The second mass spectrometer induces collisions between the chosen ions or molecules and the collision gas, often helium or argon, to produce fragmentation. The terms "collision induced dissociation" (CID) and "collision activated dissociation" (CAD) describe this phenomenon.

LC-MS

The benefits of LC separation and MS identification are combined in this method.35,36 Components are analyzed by mass spectrometer as they elute from the mixture, as shown in Fig. 4.

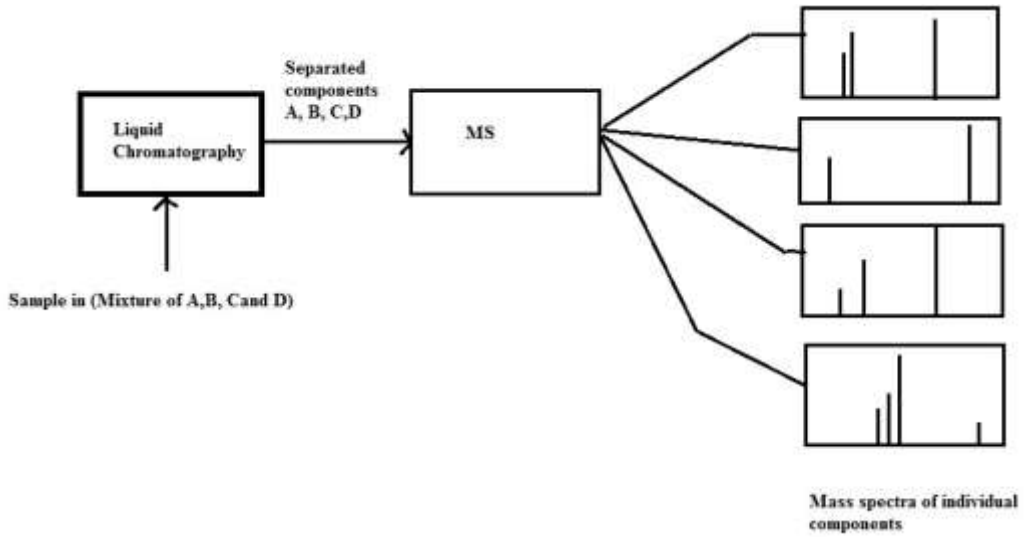

Figure 4 Application of LC-MS in separation followed by qualitative analysis.

Within a short time, the components of the separated mixture elute. Ion source component concentration begins at zero, rises to its maximum, and then falls back to zero. When the speed of the ions traveling through the ion source exceeds the scanning speed of the mass spectrometer, false spectra are obtained. Therefore, a fast scan of the spectrum is required to ensure that the concentration of eluting species is not significantly altered throughout the acquisition process. In LC-MS research, quadrupoles are therefore commonly employed. Even while magnetic sector instruments scan more slowly than quadrupole ones, they provide superior mass resolution. The quality of the mass spectrum produced by an ion source is proportional to the amount of the material used to create it. This means that the optimal moment to perform a scan is right at the peak concentration (the highest point of the LC curve). It is time consuming to keep an eye on the chromatogram constantly when it is in the development phase. As a result, the mass spectrum is scanned constantly (within the range of 50-500 mass units) at regular intervals (for instance, once every 10 seconds). This allows for the recording of a mass spectrum in the hundreds. By scanning the electric current, the computer calculates the total ion current, which is plotted on the y-axis, and the elution time, which is plotted on the x-axis. All of the constituents of a mixture are clearly eluted.

LC-MS's widespread adoption can be attributed to its user-friendliness, sensitivity, and ability to characterize impurities and DPs with absolute certainty. To obtain necessary medicine and product approvals, it is necessary to characterize such a minimal or trace analyte. Molecular

ions and their daughter ions' spectra of drugs and related chemicals can be generated with LC-MS. Studies involving hydrogen/deuterium exchange mass spectrometry require it for the measurement of masses, RBD (ring plus double bond), elemental formula, and number of nitrogens, and the identification of the number of labile H.36 The method does provide some difficulties, though.

Acquisition and analysis of mass spectra

1. It is crucial to pinpoint the molecular ion peak. The peak abundance of molecular ions may be low because of
2. Trace levels of analyte (IMPs and DPs).
3. Inadequate ionization of targeted compounds in the source.
4. Unstable nature of analyte resulting in insource fragmentation.
5. Adduct formation.

It's important to catalog and actively seek out potential DP/IMP structures. Drugs and DPs/IMPs also form adduct with K^+, Na^+, NH_4^+ and can be picked up using a positive ionization technique. In negative ionization mode, you can also detect an adduct with chloride, acetate, or formate. Sodium and potassium adducts may occur in addition to the lines caused by the noncovalent dimer/trimer of the analyte.

Elemental composition: Exact mass refers to theoretical mass, while accurate mass refers to experimental mass. A little discrepancy in precise mass can be expected from one measuring device to the next. It can be used in the determination of chemical formulae. It's a purely computational problem.

Nitrogen rule: It shows if there are more nitrogen molecules of odd or even mass. The nitrogen rule changed direction between ESI and APCI.

IHD (Index of Hydrogen Deficiency) / RDB (Ring plus Double Bond)

$$IHD = X - Y/2 + Z/2 + 1$$

Where (X, Y, Z) represents the total number of carbons, monovalent atoms, and trivalent atoms, respectively. It makes no difference how many divalent ions there are. IHD values for radical ions and simple molecules are integers. This number is a noninteger multiple of 0.5, making it applicable to quasi molecular ions and fragment ions. Structure verification by RDB and formula may fail if multivalent components are present.

Mass error calculation: There is a discrepancy between the theoretically exact mass and the experimentally precise mass. Instead of using parts per million (ppm), this inaccuracy should be expressed in millimilliunits (mmu).

$$mmu = 10^3 \text{ (Measured mass-Theoretical mass)}$$

$$ppm = \frac{10^6 \text{ (Measured mass-Theoretical mass)}}{\text{Theoretical mass}}$$

For big masses, the ppm error decreases whereas it increases for tiny masses. To limit the possible elemental formulas for a given accurate mass, a mass error (tolerance/ restriction) can be applied. Errors expressed in millimeters of mercury (mmu) allow for more precise calculations of given strikes. The ratio of naturally occurring isotopes determines the intensity of extra lines that result from isotopic abundance.

CONCLUSION

R-Lacosamide from human plasma in the presence of S-Lacosamide, a direct chiral HPLC-tandem MS technique in multiple reaction monitoring (MRM) mode was developed using liquid-liquid extraction and chiral stationary phase. From 100 L of human plasma treated with K2-EDTA as an anticoagulant, methyl tert-butyl ether was used to extract R-lacosamide, S-lacosamide, and the internal standard R-lacosamide-d6. To separate the two enantiomers, we used a diacel, chiralpak IC-3 column with a mobile phase of 100% acetonitrile flowing at a rate of 1.0 ml/min. Retention times of 6.20+0.5 and 8.00+0.5 min were obtained for R and S-lacosamide, respectively, indicating that both enantiomers resolve well. From 100.041 to 15008.054 ng mL-1, linearity was seen with the technique. Recovery, matrix effect, specificity, precision, accuracy, carryover, haemolysis and lipolysis effect, dilution integrity, and several types of stabilities were all verified during the validation process. Human plasma Rlacosamide analysis by the developed chiral HPLC-tandem bioanalytical approach is feasible. This technique is essential for maintaining therapeutic plasma concentrations of R-lacosamide. The approach is sensitive enough to quantify R-lacosamide in human plasma even in the presence of S-lacosamide.

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