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Review Article

A Review On Analytical Methods For Estimation Of Naloxone Hcl And Buprenorphine Hcl In Pharmaceutical Dosage Form

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ABSTRACT

This review article represents the collection and discussion of various analytical methods available in the literature for the simultaneous estimation of Naloxone and Buprenorphine in pharmaceutical and biological samples consisting of HPLC, UV-visible method, near-IR spectroscopy, spectrofluorometry, and hyphenated techniques such as LC-MS, LC-MS/MS, UPLC-MS/MS, and GC-MS. The anticipated review provides details about the comparative utilization of various analytical techniques for the determination of Naloxone and Buprenorphine. When it comes to offering solutions like development, analytical techniques are crucial. An overview and classification of the many analytical techniques used to identify supply issues will be provided in this article. In the quality assurance and internal control of the majority of pharmaceutical medications and preparations, pharmaceutical analysis plays a special function. The need for innovative analytical methods in the pharmaceutical business has increased as a result of the fast development of the pharmaceutical and pharmaceutical industries in many regions of the world. So, developing one's analytical abilities has become a beneficial learning process. Analytical equipment improvements have led to recent breakthroughs in analytical procedures.

INTRODUCTION

Naloxone is an opioid receptor antagonist which is used to quickly cure an opioid overdose. Additionally present as an abuse deterrent to stop injection in some medication formulations. An opioid antagonist medicine called naloxone is used to prevent or reverse the effects of opioid medications, especially when they are overdoses, which are now the top cause of death globally.[1] More precisely, naloxone works as an inverse agonist at -opioid receptors, where it has a high

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affinity, quickly flushing out any other medications attached to these receptors. When used to treat severe pain, buprenorphine is a weak partial mu-opioid receptor agonist and a weak kappa-opioid receptor antagonist. As a frequent substitute for methadone in the treatment of severe opioid addiction, it is also widely utilised. Combining Suboxone with naloxone is meant to lower its potential for misuse because naloxone is poorly absorbed when taken orally (and has no impact when done so), but if administered intravenously, it would negate the opioid agonist effects of buprenorphine. [2,3]

Analytical method development:

Analytical techniques like spectrometry and chromatography are employed in the pharmaceutical business for the qualitative and quantitative analysis of medicines, API, raw materials, and biological samples. These techniques are employed to guarantee the identification, purity, potency, and effectiveness of pharmaceuticals. The creation of analytical methods is essential to the creation and production of medications. [4]

Every year, more medications are introduced to the market. These medications may be newly developed or may only include minor structural changes to existing ones. The time between a drug's introduction to the market and its inclusion in pharmacopoeias typically elapses. This happens as a result of the challenger's ongoing and widespread medication research efforts, reports of new toxicities, the increasing patient resistance, and the potential unpredictability of their usage. As a result, it's possible that the pharmacopoeias don't have standards and analytical techniques for these medications. Therefore, it was necessary to create the most modern analytical techniques for such drugs. The scope of analytical method development often includes a thorough explanation of the analytical process. [5]

A proper analytical technique for the pharmaceuticals must not be documented in the literature owing to patent laws, and the drug or medications should not be listed as official in any pharmacopoeia. These are the basic prerequisites for novel method creation for drug analysis.[6]

There must be no existing analytical techniques for a medication when combined with other pharmaceuticals, and the current analytical approach may call for pricey reagents and solvents.[7]

There are several steps included in method development:

Physiochemical properties of drugs

- Setting up instrumental conditions
- Analyte extraction
- Sample preparation
- Method improvement
- Method validation

Validation of analytical method:

The purpose of validation is to show that any activity, technique, equipment, material, or procedure works as predicted under a certain set of conditions. The method through which it is created is based on laboratory research showing how the performance requirements of the method relate to the demand for purposeful analytical application. The analytical process describes how to do the analysis. It provides information on each step needed to carry out all analytical tests. An analytical technique is validated to show that it is acceptable for the use for which it is designed. The identification test, quantitative test, limit test, and qualitative test are the four primary types of method to be validated. Analytical technique was designed and validated utilising a variety of factors in accordance with ICH Q2 (R1) guidelines. The process of developing a method is both somewhat and highly challenging. To generate exact and accurate separation for any activities, method development laboratories need to estimate a

combination of mobile phase, temperature, pH, column, and gradient. [8,9]

The characteristics of analytical method validation are:

- Accuracy
- Specificity
- Linearity
- Range
- Precision
- Limit of detection
- Limit of quantification
- Robustness

A] Name- Naloxone Hydrochloride [10,11]

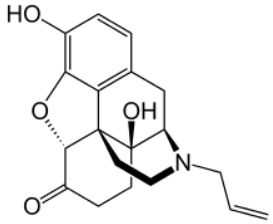

Fig. 1. Structure of Naloxone Hydrochloride

DRUG PROFILE:

Drug	**Naloxone Hydrochloride**
IUPAC Name	(1S,5R,13R,17S)-10,17-dihydroxy-4-(prop-2-en-1-yl)-12-oxa-4-azapentacyclo[9.6.1.0^{1,13}.0^{5,17}.0^{7,18}]octadeca-7,9,11(18)-trien-14-one hydrochloride
Chemical Formula	$C_{19}H_{22}ClNO_4$
Molecular Mass	363.83 g/mol
Solubility	Soluble in water (73 mg/ml), ethanol (3.3 mg/ml), methanol (50 mg/ml) and dimethyl sulfoxide (73 mg/ml)
pKa	8.39
LogP	2.09
Melting Point	184-186^{0}C
Therapeutic Use	Naloxone is an opioid receptor antagonist used to rapidly reverse an opioid overdose.

PHARMACOLOGY

Indication:

The immediate treatment of an opioid overdose or suspected opioid overdose is advised for naloxone nasal sprays. For the whole or partial reversal of opioid depression, the diagnosis of known or suspected opioid overdose, and as an adjuvant therapy in the treatment of septic shock, intramuscular, intravenous, and subcutaneous injections are advised.

Pharmacodynamics:

An opioid receptor antagonist called naloxone is used to reverse opioid overdoses. Compared to opioids, naloxone acts more quickly, thus numerous doses could be needed. Naloxone has a broad therapeutic window since it is ineffective in patients who have not taken opioids. Naloxone users may experience opioid withdrawal, and those who give the treatment should be aware that, in cases when other substances are also present, reversing opioid overdoses may not completely alleviate all of the patient's symptoms.

Mechanism of action:

The -opioid receptor is competitively inhibited by naloxone. The effects of opioids are reversed by naloxone, which blocks their activity. Naloxone does not significantly affect people who have not taken opioids.

PHARMACOKINETIC:

- **Absorption:** The bioavailability of an intranasal dosage of naloxone is 42-47%. 7 With a Cmax of 12.3–12.8 ng/mL, a Tmax of 0.25 hours, and an AUC of 16.7–19.0 h*ng/mL, an 8 mg dosage of nasal naloxone achieves these results. 7 An intramuscular dosage of 0.4 mg results in Cmax values between 0.876 and 0.910 ng/mL, a Tmax of 0.25 hours, and an AUC of 1.94 to 1.95 h*ng/mL. 7 A 2 mg intravenous dosage results in an AUC of 12.8 h*ng/ml and a Cmax of 26.2 ng/mL.

- **Volume of distribution:** Naloxone is distributed in a 200 L container. Naloxone quickly spreads throughout tissues. The blood-brain barrier and the placenta may both be crossed by it.
- **Metabolism:** To create naloxone-3-glucuronide, naloxone predominantly undergoes glucuronidation. Besides being 6-keto reduced to naloxol, naloxone may also be N-dealkylated to noroxymorphone.
- **Protein Binding:** Naloxone is significantly bound to other proteins but is only 45% bound to albumin.
- **Route of elimination:** Naloxone is 25–40% removed in the urine within 6 hours of oral or intravenous administration, 50% within 24 hours, and 60–70% within 72 hours. Urine tests show the presence of the metabolite's naloxone-3-glucuronide, noroxymorphone, and naloxol.

B] Name- Buprenorphine Hydrochloride [12,13]

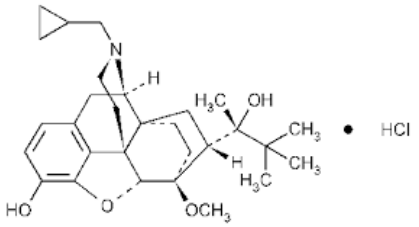

Fig. 2. Structure of Buprenorphine Hydrochloride

DRUG PROFILE:

Drug	**Buprenorphine Hydrochloride**
IUPAC Name	(1S,2R,6S,14R,15R,16R)-3-(cyclopropylmethyl)-16-[(2S)-2-hydroxy-3,3-dimethylbutan-2-yl]-15-methoxy-13-oxa-3-azahexacyclo[13.2.2.1^{2,8}.0^{1,6}.0^{6,14}.0^{7,12}]icosa-7,9,11-trien-11-ol hydrochloride
Chemical Formula	$C_{29}H_{42}ClNO_4$
Molecular Mass	504.10 g/mol
Solubility	A white or nearly white powder is buprenorphine. It dissolves very slightly in water, easily in acetone, methanol, and very slightly in cyclohexane. It also dissolves in diluted acid solutions.
pKa	8.5
LogP	4.98
Melting Point	217-219 ^{0}C
Therapeutic Use	Used for the treatment of opioid addiction.

PHARMACOLOGY

Indication:

Buprenorphine is used to relieve pain that is too severe to be managed with other therapies and necessitates the use of an opioid analgesic. For the treatment of moderate to severe opioid use disorders, buprenorphine and naloxone are also utilised in a fixed-dose combination medication.

Pharmacodynamics:

The opioid mu-receptor is the primary site of interaction for buprenorphine. The human brain, spinal cord, and other tissues all include distinct distributions of these mu-binding sites. Buprenorphine's main pharmacologic effects on the central nervous system occur in clinical situations. Its main therapeutic effects are drowsiness and analgesia. Along with analgesia, mood swings, euphoria and dysphoria, and tiredness frequently happen. Buprenorphine suppresses the cough reflex, constricts the pupils, and suppresses the respiratory centres.

Mechanism of action:

At the mu-opioid receptor, buprenorphine is a partial agonist; at the kappa-opioid receptor, it is an antagonist. Despite having a lesser intrinsic activity than other complete mu-opioid agonists like heroin, oxycodone, or methadone, it has a strong affinity for the mu-opioid receptor. This indicates that buprenorphine preferentially binds the opioid receptor and displaces opioids of lesser affinity without substantially activating the receptor. Clinically, this causes a gradual beginning of action and a phenomenon known as

the "ceiling effect," in which buprenorphine's effects plateau after a particular dose is achieved. This effect can be advantageous, though, as it lowers the danger of overdose compared to methadone and other full agonist opioids, as dose-related adverse effects such respiratory depression, drowsiness, and intoxication similarly plateau at about 32mg. Additionally, it implies that patients who are opioid-dependent may not feel euphoria or sedation as quickly as they might with stronger opioids, enhancing quality of life for those who are in excruciating pain and minimising the reinforcing effects of opioids that might result in drug-seeking behaviours.

PHARMACOKINETIC:

- **Absorption:** Buprenorphine/naloxone has a very high bioavailability after intravenous or subcutaneous administration, a reduced bioavailability when supplied sublingually or buccally, and a very poor bioavailability when taken orally. As a result, it is offered as a sublingual tablet, which is absorbed from the oral mucosa and enters the bloodstream immediately.
- **Volume of distribution:** Due to its high lipophilicity and widespread distribution, buprenorphine crosses the blood-brain barrier quickly. When administered intravenously, the estimated distribution volume ranges from 188 to 335 L.
- **Metabolism:** Through N-dealkylation, which is mediated by Cytochrome P450 3A4/3A5, buprenorphine is converted to norbuprenorphine. Additionally, buprenorphine and norbuprenorphine are both glucuronidated to produce their respective inactive metabolites, buprenorphine-3-glucuronide and norbuprenorphine-3-glucuronide.
- **Protein Binding:** Naloxone is significantly bound to other proteins but is only 45% bound to albumin.
- **Route of elimination:** Buprenorphine is metabolised by the liver, same like morphine and other phenolic opioid analgesics, and its clearance is influenced by hepatic blood flow. As free forms of buprenorphine and norbuprenorphine, it is largely removed through faeces, with 10–30% of the dosage being excreted in urine (as conjugated forms of buprenorphine and norbuprenorphine).

PUBLISHED METHODS

All of these techniques are used to estimate the amounts of naloxone and buprenorphine in pharmaceutical dosage forms and commercial formulations. The literature review includes a description of the analytical methods used. Numerous U.V., NMR, MASS, IR spectroscopic, HPLC, UPLC, HPTLC, TLC, LC/MS, and other analytical procedures for the estimation of Naloxone and Buprenorphine in a single determination and its combination with other drugs have been reported. The list of techniques, circumstances, and information sources is followed by an explanation of each technique.

- ***K. Kalyani, V. Anuradha et al*** has been designed and verified to simultaneously estimate naloxone hydrochloride and buprenorphine hydrochloride in pure and commercial formulations using reverse phase high performance liquid chromatography (RPHPLC). An isocratic separation procedure was used with the column Hypersil ODS C18 (250 mm x 4.6 mm x 5 m particle size), ammonium acetate as the mobile phase, acetonitrile as the buffer (68:32 v/v), and UV detection at 310 nm. One millilitre per minute of flow was used to elute the chemicals. Naloxone and buprenorphine had average retention durations of 2.86 and 3.67 minutes, respectively. In accordance with ICH recommendations, the approach was verified. In accordance with ICH recommendations, the approach was verified. The suggested

HPLC method's high degree of accuracy and precision is indicated by the fact that the %RSD of all validation parameters was less than 2%. For Naloxone and Buprenorphine, the technique was linear for the concentration ranges of 5- 30 g/ml and 20- 120 g/ml, respectively. Naloxone's LOD and LOQ were determined to be 0.08 g/mL and 0.26 g/mL, and buprenorphine's LOD and LOQ were determined to be 0.0078 g/mL and 0.0237 g/mL. The medications were also subjected to oxidative, thermal, photolytic, acidic, alkaline, and oxidative conditions. The stressed samples were then tested using the suggested approach. Studies on drug degradation revealed that both substances were extremely stable in an acidic, oxidative, thermal, and photolytic environment. Under alkaline circumstances, RT values were altered to be lower than they would have been otherwise, with no new peaks. The method's usefulness for the simultaneous measurement of buprenorphine hydrochloride and naloxone hydrochloride in pure and commercial formulations is supported by the high percentage of stability under stress circumstances.[1]

- ***Dhanashree a. Mundhey, nidhi p. Sapkal et al*** developed and validated a simple, rapid and accurate vierordt's method or simultaneous equation (SE) method for the simultaneous estimation of Buprenorphine HCl (BU) and Naloxone HCl (NA) in bulk and pharmaceutical dosage form. The suggested technique was approved in accordance with the standards of the Association of Official Analytical Chemists International and the International Conference on Harmonization. Methods: The approach was based on a maximum absorbance measurement at two wavelengths, 289.0 nm and 283.8 nm. Results: In the concentration range of 40-200 g ml of BU and NA in methanol, respectively, calibration curves were linear. 40-260 g ml-1 for NA and -1 for BU. The average recovery, limit of quantification, and limit of detection (LOQ), respectively, for BU and NA, were 98.91%, 0.481 g ml-1 and 0.158 g ml-1. The approach proved accurate, with relative standard deviations for both medications less than 2.0%. The quantification of BU and NA was not significantly impacted by the parameters examined for robustness. [2]
- ***Effat Souria, Farzaneh Sadat Ahmadi et al*** shows that Buprenorphine is a partial mu agonist and kappa antagonist which is used for the treatment of pain and opioid addiction. For the treatment of opioid dependency, a combination of buprenorphine hydrochloride and naloxone hydrochloride has been authorised. In this work, buprenorphine hydrochloride and naloxone hydrochloride were simultaneously determined in tablets using a third order derivative spectrophotometric approach based on the zero-crossing methodology. The measurements were done on the third order derivative spectra of buprenorphine hydrochloride and naloxone hydrochloride, respectively, obtained in methanol and 0.1 M NaOH (50:50) as solvent, at wavelengths of 257.8 nm (zero-crossing point of naloxone hydrochloride) and 252.2 nm (zero-crossing point of buprenorphine hydrochloride). In the range of 20–80 g/mL for buprenorphine hydrochloride and 5–20 g/mL for naloxone hydrochloride, the technique was discovered to be linear. In the range of 20–80 g/mL for buprenorphine hydrochloride and 5–20 g/mL for naloxone hydrochloride, the technique was discovered to be linear. Less than 2.5% and 1.8%, respectively, were the coefficients of variation and error for within-day and between-day data. Without interference from

excipients or the requirement for previous separation before analysis, the suggested approach was effectively employed to determine these medicines in pharmaceutical dosage form simultaneously.[3]

- ***Dr. Gampa Vijay Kumar, G. Sravanya et al*** carried out the estimation of Buprenorphine and Naloxone was by RP-HPLC. The mobile phase was optimised using methanol:phosphate buffer combined at a ratio of 70:30% v/v, and the phosphate buffer had a pH of 3.0. The stationary phase was an inert C18 column C18 (4.6 x 150mm, 5 m) or an equivalent chemically attached to porous silica particles. A UV detector operating at 260 nm was used for the detection. Chromatography of the solutions was done at a steady flow rate of 0.8 ml/min. Buprenorphine and naloxone both have linearity ranges between 100 and 500 g/ml and 1 to 5 g/ml, respectively. The linear regression coefficient was 0.999 or less. The values of% RSD are less than 2%, demonstrating the method's accuracy and precision. Between 98 to 102% of buprenorphine and naloxone are recovered. The linear regression coefficient was 0.999 or less. The values of% RSD are less than 2%, demonstrating the method's accuracy and precision. Between 98 to 102% of buprenorphine and naloxone are recovered. LOD and LOQ were discovered to be within tolerance. The validation parameters' findings were in compliance with ICH and USP standards. It implied that the process was straightforward, accurate, exact, and linear. The technique was discovered to be highly accurate and precise, making it appropriate for use in normal laboratory analysis. [4]
- ***Krishna Sarma Pathy et al*** developed A reverse phase high performance liquid chromatographic (RPHPLC) method and validated for simultaneous estimation of Naloxone Hydrochloride and Buprenorphine Hydrochloride in pure and marketed formulations. Using a column called Hypersil ODS C18 with particle sizes of 250 mm x 4.6 mm x 5 m and a mobile phase made up of ammonium acetate with a pH of 6.0, separation was performed. Acetonitrile (68:32) v/v and UV detection at 310 nm serve as the buffer. A flow rate of 1 mL per minute was used to elute the chemicals. Naloxone and buprenorphine had average retention durations of 2.86 and 3.67 minutes, respectively. In accordance with ICH recommendations, the approach was verified. The percentage RSD of all validation parameters was found to be less than 2%, demonstrating the proposed HPLC method's high degree of accuracy and precision. For Naloxone and Buprenorphine, the method was linear between concentrations of 5.30 g/ml and 20–120 g/ml, respectively. Naloxone's LOD and LOQ were determined to be 0.08 g/mL and 0.26 g/mL, and buprenorphine's LOD and LOQ were determined to be 0.0078 g/mL and 0.0237 g/mL. The medications were also subjected to oxidative, thermal, photolytic, acidic, alkaline, and oxidative conditions. The stressed samples were then tested using the suggested approach. Studies on drug degradation revealed that both substances were extremely stable in an acidic, oxidative, thermal, and photolytic environment. Under alkaline circumstances, RT values were altered to be lower than they would have been otherwise, with no new peaks. The method's usefulness for the simultaneous measurement of buprenorphine hydrochloride and naloxone hydrochloride in pure and commercial formulations is supported by the high percentage of stability under stress circumstances. [5]

- ***Vidhi N. Patel, Mitali H. Jasani et al*** carried-out A simple, precise and accurate stability indicating RP-HPLC method for estimation of Buprenorphine HCl (BH) and Naloxone HCl Dihydrate (NHD). A C18 column (250 x 4.6 mm, 5 m) was used for the validation, and the mobile phase was made up of acetonitrile and buffer at a ratio of 75:25 that was degassed using sonication. The flow rate was changed to 1 mL/min, and a PDA detector was used to measure the effluent at 273 nm. Buprenorphine hydrochloride and Naloxone hydrochloride dihydrate had retention times of 8.198 and 3.113 minutes, respectively. The method's linearity, precision, accuracy, and specificity, as well as its limits of detection and quantization, were all verified. Buprenorphine hydrochloride and Naloxone hydrochloride dihydrate both have linearities of 40-140 g/ml and 160-560 g/ml, respectively. The percentages of both medicines recovered from the synthetic formulation were determined to be 100.79% for buprenorphine hydrochloride and 100.42% for dihydrate naloxone. The degradation of buprenorphine HCl and naloxone HCl dihydrate was below acceptable limits. During the investigation, it was discovered that the procedure was exact, accurate, and specific. The technique can be utilised for routine examination of commercially available goods containing a synthetic combination of buprenorphine hydrochloride and naloxone hydrochloride dihydrate. [6]
- ***Katasani Damodar et al*** shows that the developed method is selective, linear, precise and accurate for the simultaneous estimation of Buprenorphine hydrochloride and Naloxone hydrochloride in pharmaceutical dosage forms. On a symmetrical C18 column, isocratic elution was used at a flow rate of 1 ml min-1 at room temperature. Ortho phosphoric acid, methanol, and water in the proportions of 60:20:20 (v/v/v) made up the mobile phase. The wavelength used for UV detection was 273 nm. Naloxone hydrochloride had a retention duration of 5.85 min and buprenorphine hydrochloride of 3.85 min. The technique was approved in accordance with ICH standards. For the accurate calculation of buprenorphine hydrochloride and naloxone hydrochloride in pharmaceutical dosage forms, the suggested approach can be used. [7]
- ***Irina Dioumaeva et al*** shows that determination of buprenorphine and norbuprenorphine in whole blood by forensic toxicology laboratories requires an analytical method capable of reliable detection of these compounds at concentrations below 1 ng/mL. A simple sample cleanup procedure coupled with an LC/MS/MS method using mass transitions 468.2 & 55.1 and 414.2 & 83.1 allows for a limit of detection (LOD) below 0.1 ng/mL for both analytes. Typical calibration curves are linear in the range of 0.2 to 20 ng/mL for each analyte, with R2 values equal or higher than 0.999. High sensitivity is achieved by using Agilent products, including an Agilent Bond Elut Plexa PCX mixed mode polymeric SPE sorbent, an Agilent Poroshell 120 EC-C18 2.7 µm superficially porous LC column, an Agilent 1200 Infinity LC system, and an Agilent 6460 Triple Quadrupole LC/MS System with Agilent Jet Stream Technology (AJST) enhanced electrospray source.[8]
- ***Marta Tikhomirov, Błazej Pozniak*** shows that the precise and reliable determination of buprenorphine concentration is fundamental in certain medical or research applications, particularly in pharmacokinetic studies of this opioid. The main challenge is, however, the

development of an analytical method that is sensitive enough, as the detected in vivo concentrations often fall in very low ranges. Thus, in this study we aimed at developing a sensitive, repeatable, cost-efficient, and easy HPLC analytical protocol for buprenorphine in rabbit plasma. In order to obtain this, the HPLC-MS2 system was used to elaborate and validate the method for samples purified with liquid-liquid extraction. Fragment ions 468.6→396.2 and 468.6→414.2 were monitored, and the method resulted in a high repeatability and reproducibility and a limit of quantification of 0.25 µg/L with a recovery of 98.7–109.0%. The method was linear in a range of 0.25–2000 µg/L. The suitability of the analytical procedure was tested in rabbits in a pilot pharmacokinetic study, and it was revealed that the method was suitable for comprehensively describing the pharmacokinetic profile after buprenorphine intravenous administration at a dose of 300 µg/kg. Thus, the method suitability for pharmacokinetic application was confirmed by both the good validation results of the method and successful in vivo tests in rabbits.[9]

- ***Sarah J. Phillips, Alison Oliveto*** shows that a rapid analytical method has been developed to determine the gabapentinoid, gabapentin, and partial opioid agonist, buprenorphine in 20 microliters of human serum using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a chromatographic run time of 2 minutes. A simplified sample cleanup procedure using methanol precipitation of serum proteins/lipids followed by evaporation and reconstitution in mobile phase was demonstrated. Gabapentin and buprenorphine were detected following positive ion electrospray ionization using multiple-reaction-monitoring. The internal standard approach was used for quantitation with labeled gabapentin-D10 and buprenorphine-D4 serving as internal standards. Using organic reaction principals and stable isotope labels, collision-induced fragmentation mechanisms for both gabapentin and buprenorphine are proposed. The method was validated according to the FDA Guidance for Industry – Bioanalytical Method Validation.[14]
- ***Dhanashree Arun Mundhey, Vishal V. Rajkondawar*** shows that a development and validated of a simple, selective RP-HPLC method for the determination of buprenorphine hydrochloride in pharmaceutical microemulsion formulation. A forced degradation study of developed formulation was carried out in accordance with International Conference on Harmonization (ICH) guidelines Q1 (R2). The chromatogram was obtained with 10 mmolL−1 potassium phosphate buffer adjusted to pH 6.0 with triethanolamine and acetonitrile (17:83, v/v) as mobile phase, C18 HPLC column (250 × 4.6 mm i.d., 5 µm) kept at 30◦C and UV detection at 284 nm. The compound was eluted isocratically at a flow rate of 1.0 mL min−1 . The average retention time for buprenorphine was 14.319 min. The method was validated according to the ICH guidelines. The validation characteristics included accuracy, precision, linearity, range, specificity, limit of Quantitation and robustness. The calibration curves were linear (R2 > 0.999) over the concentration range 1.0 – 500.0 µgmL−1 for buprenorphine hydrochloride and recovery study for the compound was above 95 %. No spectral or chromatographic interferences from the microemulsion excipients were found. The drug was found to be labile under oxidative

stress condition; whereas stable under all other stress conditions.[15]

- ***Naser F Altannak*** shows that a naloxone is an opioid antagonist indicated for central nervous system and respiratory depression treatment induced by natural or synthetic opioid in adults and neonates whose mothers have received opioids. Although naloxone hydrochloride has been reported to be physically and chemically stable, photostability of naloxone hydrochloride under artificial light, and sunlight has not been reported. Therefore, a method was required for assessment of naloxone hydrochloride photostability. A high-performance liquid chromatography/mass spectrometry method was established to evaluate the photostability of naloxone hydrochloride. Injections of naloxone hydrochloride in 0.9% sodium chloride were exposed to artificial light and sunlight at room temperature for 192 hrs. Naloxone losses up to 5.26% of its initial concentration when exposed to artificial light at room temperature for 192 hrs, but the degradation increased up to 15.08% under sunlight exposure at room temperature for 192 hrs. The disappearance of naloxone hydrochloride was correlated with the appearance of noroxymorphone degradant. Conclusion: Naloxone hydrochloride is photosensitive and degradation increased as the light intensity increased. Therefore, naloxone intravenous infusion solutions should either be protected from light and/or be frequently replaced when being administered to patients.[16]
- ***Limon Khatun Nahar, Rebecca Andrews and Sue Paterson*** shows that A highly sensitive and fully validated method was developed for the quantification of buprenorphine in postmortem blood. After a twostep protein precipitation process using acetonitrile, buprenorphine was purified using mixed-mode (C8/cation exchange) solid-phase extraction cartridges. Endogenous water-soluble compounds and lipids were removed from the cartridges before the samples were eluted, concentrated and derivatized using N-methyl-N-trimethylsilyl trifluoroacetamide. The samples were analyzed using two-dimensional gas chromatography –mass spectrometry (2D GC –MS) in selective ion-monitoring mode. A low polarity Rxi-5MS (30 m 3 0.25 mm I.D. 3 0.25 mm) was used as the primary column and the secondary column was a mid-polarity Rxi -17Sil MS (15 m 3 0.32 mm I.D. 3 0.25 mm). The assay was linear from 1.0 to 50.0 ng/mL (r 2 > 0.99; n 5 6). Intraday (n 5 6) and interday (n 5 9) imprecisions (percentage relative standard deviation, % RSD) were and the average recovery was 60%. The limit of detection (LOD) of the method was 0.5 ng/mL and limit of quantification was 1.0 ng/mL. 2D GC –MS improved the LOD of buprenorphine by 20-fold compared with analysis on a conventional GC–MS. The method was highly selective with no interference from endogenous compounds or from 62 commonly encountered drugs. To prove method applicability to forensic postmortem cases,14 authentic postmortem blood samples were analyzed. [17]
- ***Xiaoyue Shan, Chengjian Cao*** shows that the abuse of buprenorphine and methadone has grown into a rising worldwide issue. After their consumption, buprenorphine, methadone and their metabolites can be found in the human organism. Due to the difficulty in the assessment of these compounds by routine drug screening, the importance of developing highly sensitive analytical approaches is undeniable. Liquid chromatography tandem mass spectrometry is the preferable technique

for the determination of buprenorphine, methadone and their metabolites in biological matrices including urine, plasma, nails or oral fluids. This research aims to review a critical discussion of the latest trends for the monitoring of buprenorphine, methadone and their metabolites in various biological specimens. [18]

- ***Ankit Rochani, PhD; Vinh Nguyen*** shows that a stability-indicating LC-MS method was developed to map the potential degradation peaks of buprenorphine when exposed to acidic, basic, and oxidative conditions. This method was used to study the stability of compounded buprenorphine oral syringes stored under refrigeration (2°C–8°C) and room temperature (25°C ± 2°C with 60% relative humidity). Syringes from each storage condition were assessed for stability using pH meter and stability-indicating LC-MS assay for 30 days. Buprenorphine gets completely degraded in the presence of acid at the end of 1 hour of exposure. Various degradation peaks were identified using LC-MS assay for buprenorphine under acidic, basic, and peroxide conditions. Stability study of oral buprenorphine syringes showed no precipitation, cloudiness, or color change during this study at all storage conditions. The LC-MS assay revealed that buprenorphine oral syringes retained greater than 90% of the initial concentrations for 30 days.[19]

CONCLUSION

In this study, naloxone and buprenorphine are categorised according to their known and unknown amounts in pharmaceutical items using a brand-new, exact analytical approach. The study includes analytical techniques for the examination of tablet dosage forms as well as bulk supplies of buprenorphine and naloxone. Several techniques for the creation and validation of various medications were discovered after a review of the literature. The many analytical techniques used to examine naloxone and buprenorphine are covered in this article. It has been researched using medicinal dosage forms, bulk LC-MS/MS, HPLC, and UPLC. In relation to these methods, drug development and validation have been studied. This study sheds light on the characteristics of buprenorphine and naloxone, which will help with the future development of analytical methods for this substance.

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