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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ROPIVACAINE: A REVIEW OF UV AND HPLC TECHNIQUES

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ABSTRACT

Ropivacaine hydrochloride is a widely used long-acting local anesthetic that requires reliable analytical methods to ensure its quality, safety, and therapeutic efficacy. Numerous analytical techniques have been reported for its quantitative determination, among which UV spectrophotometry and reverse-phase high-performance liquid chromatography (RP-HPLC) are the most commonly employed. Previous reported methods for stability-indicating forced degradation studies was not performed. Therefore, a new stability indicating RP-HPLC method was going to develop and validate. The new stability indicating RP-HPLC method is accurate, precise, reliable, and suitable for routine analysis and stability studies of Ropivacaine Hydrochloride in pharmaceutical formulations. There is No UV spectroscopy method was not done in literature review. So I plan to do UV spectroscopy method by using simple solvent in e.g Distilled water. The reported methods were compared based on chromatographic conditions, sensitivity, specificity, precision, accuracy, and compliance with ICH validation requirements. The review demonstrates that UV spectrophotometric methods are suitable for rapid routine analysis, whereas RP-HPLC offers superior performance for stability studies, impurity profiling, and regulatory quality control. overall, this review provides a comprehensive evaluation of the reported analytical methods for ropivacaine hydrochloride and serves as a valuable reference for method selection, validation, pharmaceutical development, and quality control.

1. INTRODUCTION[1,2,3,4]

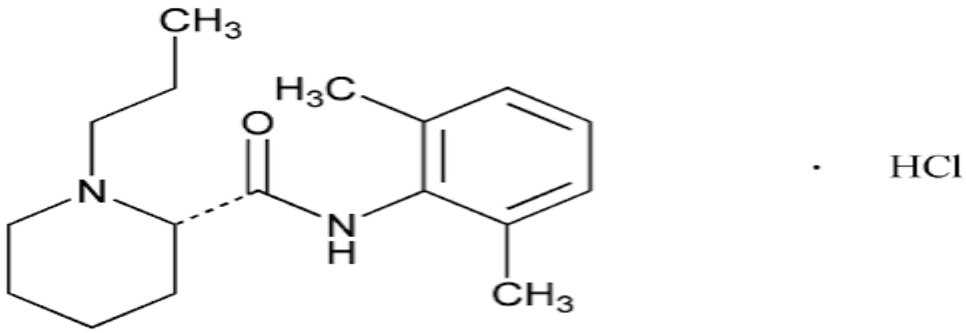
Ropivacaine is a long-acting amide-type local anesthetic that is widely used in clinical practice for surgical anesthesia, postoperative pain management, epidural anesthesia, peripheral nerve blocks, and obstetric analgesia. It is the pure S(-)-enantiomer of propivacaine and was developed to provide effective local anesthesia with reduced cardiovascular and central nervous system toxicity compared with bupivacaine. Ropivacaine has become one of the preferred local anesthetics in modern anesthetic practice.

The quality, safety, and efficacy of pharmaceutical products containing ropivacaine depend on accurate and reliable analytical methods for its identification and quantitative estimation. Analytical techniques are essential throughout the drug development process, including raw material testing, formulation development, stability studies, process validation, and routine quality control. Regulatory authorities also require validated analytical methods that comply with International Council for Harmonisation (ICH) guidelines to ensure consistent product quality and patient safety.

Among the various analytical techniques available, UV-visible spectrophotometry and reverse-phase high-performance liquid chromatography (RP-HPLC) are the most frequently employed for the determination of ropivacaine in bulk drug substances and pharmaceutical dosage forms. UV spectrophotometric methods are simple, rapid, economical, and suitable for routine analysis when high specificity is not required. In contrast, RP-HPLC offers superior sensitivity, specificity, accuracy, and precision, making it particularly useful for the analysis of complex pharmaceutical formulations, impurity profiling, and

stability-indicating studies. Both techniques have been extensively utilized for method development and validation in accordance with ICH guidelines.

Over the past decade, numerous analytical methods have been reported for the estimation of ropivacaine using UV spectrophotometry and RP-HPLC with different chromatographic conditions, detection wavelengths, mobile phase compositions, and validation approaches. A critical review of these methods is valuable for understanding current analytical practices, identifying methodological advancements, and selecting appropriate techniques for pharmaceutical quality control.



ROPIVACINE HYDROCHLORIDE

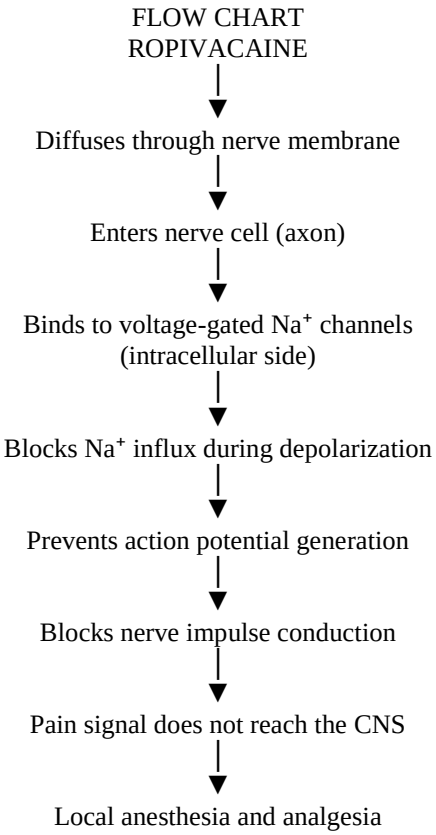
1.1 Physicochemical Properties of Ropivacaine Hydrochloride[11,12]

Property	Description
Generic Name	Ropivacaine Hydrochloride
IUPAC Name	(S)-N-(2,6-Dimethylphenyl)-1-propylpiperidine-2-carboxamide hydrochloride
Chemical Formula	$C_{17}H_{26}N_2O \cdot HCl$
Molecular Formula (Free Base)	$C_{17}H_{26}N_2O$
Molecular Weight	310.86 g/mol (Hydrochloride); 274.40 g/mol (Free Base)
Drug Class	Amide-type local anesthetic
Stereochemistry	Pure S(-)-enantiomer
CAS Number	98717-15-8 (Hydrochloride)
Appearance	White to off-white crystalline powder
Odour	Odourless
Taste	Slightly bitter
Solubility	Freely soluble in water; soluble in methanol; sparingly soluble in ethanol
pKa	Approximately 8.1
Log P	Approximately 2.9 (Free Base)
Melting Point	Approximately 143–147°C (Hydrochloride)
pH of Injection	4.0–6.0
Dosage Form	Sterile solution for injection/infusion
Available Strengths	0.2%, 0.5%, 0.75%, and 1.0% (2, 5, 7.5, and 10 mg/mL)
Storage Conditions	Store at 20–25°C; protect from excessive heat and freezing

Mechanism of Action of Ropivacaine[13,14]

Ropivacaine is a long-acting amide-type local anesthetic that produces reversible loss of sensation by blocking the conduction of nerve impulses. It acts primarily by inhibiting voltage-gated sodium (Na^+) channels located on the neuronal cell membrane. Following administration, ropivacaine diffuses through the nerve sheath and axonal membrane in its uncharged form. Inside the neuron, it becomes protonated and binds to the intracellular portion of voltage-gated sodium channels.

Binding of ropivacaine stabilizes the sodium channel in its inactivated state and prevents the influx of sodium ions during membrane depolarization. As a result, the initiation and propagation of action potentials are blocked, preventing transmission of pain signals from the peripheral nerves to the central nervous system. This produces reversible local anesthesia and analgesia. Ropivacaine preferentially blocks sensory nerve fibers over motor fibers at lower concentrations, resulting in effective pain relief with relatively less motor blockade. Compared with bupivacaine, ropivacaine has lower lipid solubility and reduced affinity for cardiac sodium channels, contributing to its lower potential for cardiotoxicity and central nervous system toxicity.



1.2. Available Formulation For Ropivacaine[21,22,23]

Drug Name (Generic)	Brand Name	Strength	Dosage Form	Original Manufacturer/Marketing Company
Ropivacaine Hydrochloride	**Naropin®**	2 mg/mL (0.2%)	Injection	AstraZeneca (original developer); marketed in several countries by Fresenius Kabi or regional license holders.
Ropivacaine Hydrochloride	**Naropin®**	5 mg/mL (0.5%)	Injection	AstraZeneca/Fresenius Kabi.
Ropivacaine Hydrochloride	**Naropin®**	7.5 mg/mL (0.75%)	Injection	AstraZeneca/Fresenius Kabi.
Ropivacaine Hydrochloride	**Naropin®**	10 mg/mL (1%)	Injection	AstraZeneca/Fresenius Kabi.

Common commercial presentations

Strength	Pack Size
0.2% (2 mg/mL)	10 mL, 20 mL, 100 mL, 200 mL
0.5% (5 mg/mL)	20 mL, 30 mL, 100 mL, 200 mL
0.75% (7.5 mg/mL)	20 mL
1.0% (10 mg/mL)	10 mL, 20 mL

Pharmacokinetic and pharmacodynamic of Ropivacaine

Pharmacokinetics of Ropivacaine[15]

- **Absorption:** Well absorbed after epidural, peripheral nerve block, and local infiltration.
- **Distribution:** Approximately **94% protein bound**, mainly to α1-acid glycoprotein.
- **Metabolism:** Extensively metabolized in the liver by **CYP1A2** and **CYP3A4** enzymes.
- **Metabolites:** Converted into inactive metabolites.
- **Excretion:** Mainly eliminated through the kidneys in urine.
- **Elimination half-life:** Approximately **1.8–6 hours** (depending on route and patient factors).

Pharmacodynamics of Ropivacaine[16]

- **Drug class:** Long-acting amide-type local anesthetic.
- **Mechanism:** Blocks **voltage-gated sodium (Na^+) channels**.

- **Action:** Prevents sodium ion influx and blocks nerve impulse conduction.
- **Clinical effect:** Produces reversible local anesthesia and analgesia.
- **Selectivity:** Preferentially blocks sensory fibers over motor fibers.
- **Advantage:** Lower cardiotoxicity and central nervous system toxicity compared with bupivacaine.
- **Clinical uses:** Epidural anesthesia, peripheral nerve block, local infiltration, and postoperative pain management.

Adverse effects[17]

Adverse drug reactions (ADRs) are rare when it is administered correctly. Most ADRs relate to administration technique (resulting in systemic exposure) or pharmacological effects of anesthesia, however allergic reactions can rarely occur.

Systemic exposure to excessive quantities of ropivacaine mainly result in central nervous system (CNS) and cardiovascular effects – CNS effects usually occur at lower blood plasma concentrations and additional cardiovascular effects present at higher concentrations, though cardiovascular collapse may also occur with low concentrations.

CNS effects may include CNS excitation (nervousness, tingling around the mouth, tinnitus, tremor, dizziness, blurred vision, seizures followed by depression (drowsiness, loss of consciousness), respiratory depression and apnea).

Cardiovascular effects include hypotension, bradycardia, arrhythmias, and/or cardiac arrest – some of which may be due to hypoxemia secondary to respiratory depression.

REPORTED METHOD

1. Simultaneous determination of procaine, lidocaine, ropivacaine, tetracaine and bupivacaine in human plasma by high-performance liquid chromatography. It was achieved on C18 column with a mobile phase consisting of 30 mM potassium dihydrogen phosphate buffer (0.16% triethylamine, pH adjusted to 4.9 with phosphoric acid) and acetonitrile (63/37, v/v). The detection was performed simultaneously at wavelengths of 210 and 290 nm.[5]
2. Simultaneous determination of three local anesthetic drugs from the pipecoloxylidide group in human serum by high-performance liquid chromatography. It was achieved by C(18) reversed-phase column, attached to a UV detector. The mobile phase was composed of acetonitrile-methanol-30 mM NaH(2)PO(4) (pH 5.6) (100: 100: 300, v/v/v) and the flow rate was 1ml/min. The absorbance at 210 nm. The retention times of 9.7min.[6]
3. Determination of free concentration of ropivacaine and bupivacaine in blood plasma by ultrafiltration and coupled-column liquid chromatography. coupled-column liquid chromatographic system, consisting of one reversed-phase and one ion-exchange column. The system was highly selective. Ropivacaine were detected by at 210 nm. The limit of determination was 10 nm and the inter-assay precision at a concentration level of about 100 nm was 6% (R.S.D., n = 30).[7]
4. HPLC method devised for the quantitative determination of ropivacaine in drug-delivery systems. The measurements at 30°C were conducted at 240 nm, at a flow rate of 1.2 ml/ min, ropivacaine (0.08 or 1.16 mM) were diluted in 20 mM HEPES buffer pH 7.0. RVC: HP-b cyclodextrin inclusion complexation Solid inclusion complexes in 1: 1 stoichiometry were obtained by mixing appropriate amounts of HPLC and RVC in water, as previously reported.[8]
5. Simultaneous determination of ropivacaine and antipyrine by high performance liquid chromatography and its application to the in vitro transplacental study. The mobile phase contains potassium dihydrogen phosphate (25 mM, adjusted to pH 5.0 with phosphoric acid)-acetonitrile (79: 21, v/v) uv detector at 210 nm.[9]
6. High-performance liquid chromatographic determination of ropivacaine,3-hydroxy-ropivacaine, 4-hydroxy-ropivacaineand2',6'pipecoloxylidide in plasma. C8 column with a mobile phase mixture of acetonitrile and pH 2.1, 0.01 M potassium dihydrogen phosphate. The extraction yields of ropivacaine were 94.7%.[10]

HPLC METHODS

METHODS	MOBILE PHASE	FLOW RATE	WAVELENGTH
LC method development and validation for the determination of Ropivacaine HCl in bulk & formulation	Monobasic phosphate buffer pH 2.5 and Acetonitrile	1.0 ml/min	220 nm
Simultaneous determination of Ropivacaine and antipyrine by HPLC and its application to the in vitro transplacental study	Potassium dihydrogen phosphate (Ph 5.0 with ortho phosphoric acid) acetonitrile (79: 21, v/v)	1.0 ml/min	210 nm
Validation of an HPLC method devised for the quantitative determination of Ropivacaine in drug -delivery systems	Acetonitrile and phosphate buffer pH 8.0 (60: 40, v\v)	1.2 ml/min	240 nm
Response surface methodology for the	Acetonitrile and methanol and	1.5 ml/min	270 nm

optimized and validated investigation of Ropivacaine in bulk and tablet dosage form by RP – HPLC	water (40: 30: 30 , v/v) with 0.1% TEA		
HPLC using UV detection for the Simultaneous quantification of Ropivacaine and Bupivacaine in human plasma	Mixture 70mm sodium sulfate (pH 3.1) and acetonitrile (70: 30)	1.5ml/min	205 nm

CONCLUSION

The reported analytical methods confirm that only RP-HPLC was done. There is no stability study. So I plan to do stability indicating RP-HPLC Method and also UV spectroscopy because still now there is no UV method available are reliable techniques for the quantitative estimation of Ropivacaine. Such as forced degradation studies under acidic, alkaline (basic), hydrolytic, thermal, photolytic and oxidative stress conditions using distilled water as the primary solvent for sample preparation. The developed method effectively separated the intact drug from its degradation products, demonstrating excellent specificity and stability-indicating capability. The validation results confirmed that the method complies with ICH guidelines with respect to linearity, accuracy, precision, robustness, sensitivity, and reproducibility. Although UV methods are simple, economical, and suitable for routine pharmaceutical analysis, RP-HPLC provides greater analytical sensitivity, specificity, and robustness for advanced quality-control and stability studies. Continued improvements in analytical technology are expected to produce faster, environmentally friendly, and more efficient methods that comply with current regulatory standards. This review provides a consolidated reference for future analytical method development and validation studies involving Ropivacaine hydrochloride.

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