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# A REVIEW ON ESTIMATION OF LOCAL ANAESTHETICS BY HIGH PERFORMANCE LIQUID CHORMATOGRAPHICMETHODS

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**ABSTRACT**

The main goal of this review is to provide overview on the Reverse phase high performance liquid chromatography and normal phase high performance liquid chromatographic methods that are necessary to analyse the local anaesthetics. Local anaesthetics are used in a wide range of clinical situations to prevent acute pain and to stop or ameliorate pain produced by cancer or pain associated with chronic painful conditions. These have similar chemical structure but differing pharmacokinetic properties and spectra of pharmacodynamics effects that influence selection of agents for use in various clinical situations. Local anaesthetics (LAs) are drugs which can applied upon topical application or local injection cause reversible loss of sensory perception, especially of pain, in a restricted area of the body. These classes of drugs are mainly analysed by using Reverse phase HPLC in which stationary phase non-polar and mobile phase is polar and Normal phase HPLC in which stationary phase is polar and mobile phase is non-polar.

**KEYWORDS:** Reverse Phase –Hplc, Hplc, Lidocaine, Bupivacaine, Local Anasthetics.

## INTRODUCTION

Local anaesthetics (LAs) are drugs which can applied upon topical application or local injection cause reversible loss of sensory perception, especially of pain, in a restricted area of the body. They block generation and conduction of nerve impulse at any part of the neurone with which they come in contact, without causing any structural damage. Thus not only sensory but also motor impulses are interrupted when a local anaesthetic is applied to a mixed nerve, resulting in muscular paralysis and loss of autonomic control.[1, 2] Local anaesthetics (LAs) are classified into two groups based on nature amides [- NH-CO-] and Esters [-O-CO]. Bupivacaine and Lidocaine are the amide forms.[3] Reversed phase mode is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non-polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complication) to control retention and selectivity. The polar compound gets eluted first in this mode and non-polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. In the normal phase mode, the stationary phase is polar and the mobile phase is non-polar in nature. In this technique, non-polar compounds travel faster and are eluted first. This is because of the lower affinity between the non-polar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

### Types of anaesthesia

- **Surface anaesthesia:** It is achieved by applying local anaesthetic action on skin or mucous membrane. Surface anaesthesia target is not the submucous layer its target is mucous in superficial layer.
- **Infiltration anaesthesia:** It is achieved by injecting the solution in the tissue, a layer after layer. It target mainly skin or profound tissues.
- **Conduction anaesthesia or Regional anaesthesi:** it is achieved by injecting the local anaesthetic solution around a nervous formation. This is known as nerve block anaesthesia and epidural anaesthesia.

a. **Nerve block anaesthesia:** This is achieved by injecting the solution near by a peripheral route of nerve.
b. **Epidural anaesthesia:** This is achieved by injecting of the local anaesthetic solution in epidural spaca[4]

**Mechanism Action of Local anaesthetics**

The nerve membrane consists of a bimolecular of Phospholipid and protein and that is punctuated by non-specific channels, one permeant to sodium ions and other to potassium ions that are controlled by voltage dependent gates. It jumps up to + 40 mV to form an action potential when depolarized. In outside of the membrane consists -70 Mv of resting potential which rises to above -55mV of the firing threshold. This is associated when potassium ion moves outwards and sodium ion inwards through their respective channels.[5]

**Classification of local anaesthetics**

| **Injectable local anaesthetics** | **Surface local anaesthetics** |
|---|---|
| 1.Low potency, short duration local anaesthetics :- Procaine, Chloroprocaine | Soluble anaesthetics:- Cocaine, Lidocaine, Tetracaine, Benoxinate, |
| 2. Intermediate potency and duration of local anaesthetics:- Lidocaine/Lignocaine, Prilocaine | b. Insoluble local anaesthetics:-Benzocaine, Butylaminobenzoate (Butamben), Oxethazine. |
| 3.High potency, long duration local anaesthetics:- Tetracaine(Amethocaine), Bupivacaine, Ropivacaine, Dibucaine or (Cinchocaine) | |

**METHOD**

**Table 1: RP - High Performance liquid chromatography methods for Lidocaine**

| S.no | Drug | Stationary phase | Mobile phase | Detector | Wavelength (nm) | Retention time Rt (min) | Flow rate (ml/min) |
|---|---|---|---|---|---|---|---|
| 1. | Beclomethasone Dipropionate, Clotrimazole, Chloramphenicol, Lidocaine[1] | ODS $C_{18}$ RP- column | **Solution A:-**Buffer (1.6 g of $CH_3COONH_2$ in to 1000 ml of Hplc grade water, add 10 ml of TEA, and adjust $P^H$ to 6.4±0.1 with dilute acetic acid )<br>**Solution B:-** Acetonitrile | UV | 254 nm | 16.2 min, 19.1 min, and 5.9min, 10.8 min. | 1.0 ml/min |
| 2. | Lidocaine Hydrochloride[2] | Ion pack Ercusn$C_{18}$ RP-column (250 × 4.5 mm i.d) | Acetonitrile : Water (20:80 v/v) with 5% acetic acid at $P^H$ 3.4 | UV | 254 nm | 19.0 min. | 1.0 ml/min |
| 3. | Hydrocortisone Acetate and Lidocaine[3] | Beckman – Coulter (150 ×4.6 mm,5µm particle size) | Methanol : Water (65 : 35 v/v) $P^H$ adjust to 2.5 with 85% 0- Phosphoric acid | UV | 250 nm | 2.63 min, 5.87 min. | 1.0 ml/min |
| 4. | Phenylephrine Hydrochloride and Lidocaine Hydrochloride[4] | Lichrosphere $RP_{18}$Column (250 × 4.6 mm, 5 µm particle size) | Acetonitrile and buffer ) $P^H$ adjust to 3 in the ratio of 25 : 75 v/v | UV | 254 nm | 3.537 min, 15.965 min. | 1.0 ml/min |
| 5. | Cetylpyridinium Chloride, Chlorocresol, Lidocaine[5] | ZORBAX Eclipse Plus $C_8$ Column (25 cm × 4.6 mm i.d.; 5 5 µm) | 0.05 % Phosphoric acid Solution : Acetonitrile : Methanol (15 : 24 ; 61 v/v) | DAD | 220 nm | 3.7 min, 2.7 min, 2.1min. | 1.0 ml/min |
| 6. | Thiomersal, Lidocaine, and Phenylepherine[6] | ZORBAX $C_{18}$ RP- column (250 × 4.6 mm, 5 µm i.d.,) | Buffer : Acetonitrile : Triethylamine (40 : 60 : 0.1 v/v) $P^H$ adjust to 6 with o – Phosphoric acid | UV | 245 nm | 5.483 min, 4.580 min, and 2.509 min. | 0.6 ml/min |
| 7. | Lidocaine Hydrochloride and Nifedipine[7] | LC – 20 AT $C_{18}$ RP-column (250 mm × 4.6 mm i.d.; 5 µm) Hypersil BDS Column. | Buffer (0.05 $KH_2PO_4$ adjust to $P^H$ 3.0 ) : Methanol (50 : 50 v/v) | SPD | 234 nm | 4.170 min, 6.530 min | 1.0 ml/min |
| 8. | Lidocaine and 2,6 – dimethyl aniline[8] | Partisil 5 ODS 3 RP - Column (250 × 4.6 mm) | Water: Methanol: Tetrahydrofuran (48.2: 48.2: 1.6 v/v) containing 0.02 M $KH_2PO_4$. | UV | 230 nm | 12 – 12.5 min and 9.5 – 10 min | 1.0 ml/min |
| 9. | Phenylephrine Hydrochloride, Lidocaine Hydrochloride and Betamethasone Valerate[9] | Merck $C_{18}$ – RP Column (250 × 4.6 mm 5 µm). | Phosphate buffer (0.01 M) and acetonitrile (46: 54 % v/v) adjust $P^H$ 7.0 (±0.05) with triethylamine. | UV | 270 nm | 2.65 min, 4.815 min and 5.421 min. | 1.5 ml/min |
| 10. | Lidocaine and Diclofenac diethylamine[10] | Sphere 100 $C_{18}$ – RP Column(250 × 4.6 mm 5 µm). | Acetonitrile : Phosphate dihydrogen (0.01 M) : Butane Sulfonic acid sodium salt (45 : 55 : 0.1 % v/v) and adjust to $P^H$ 6.8 ± 0.05 with triethylamine | UV | 261 nm | 5.7 min, and 14.025 min. | 1.0 ml/min |

**Table 2: RP-High Performance liquid chromatography methods for Bupivacaine**

| S.no | Drug | Stationary phase | Mobile phase | Detector | Wavelength (nm) | Retention time Rt (min) | Flow rate (ml/min) |
|---|---|---|---|---|---|---|---|
| 11. | Bisoprolol and Bupivacaine[11] | BDS Hypersil C 18 Column (150 × 4.6 mm, 5 µm Thermo Electron corp) | 65% water, 35% acetonitrile, 1ml/L triethylamine $P^H$ 2.5 | UV | 202 nm | 3.1 – 3.3 min, and 3.5 – 3.7 min | 1.0 ml/min |

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|---|---|---|---|---|---|---|---|
| 12. | Bupivacaine Hcl[12] | Waters RP – C18 Column (150 × 4.6 mm) | $P^H$ 6.5 buffer : acetonitrile (50 : 50 v/v) | PDA | 220 nm | 5.46 min | 1.0 ml/min |
| 13. | Bupivacaine[13] | RP – Column (125 × 4 mm i.D;) | Acetonitrile: 0.05 M sodium phosphate buffer, $P^H$ 6.0 (35: 65) and 0.05 % of diethylamine. | UV | 263 nm | 7.9 min | 1.0 ml/min |
| 14. | Bupivacaine and lidocaine[14] | RP – Column (150 × 4.6 mm i.D, 5 µm) | 0.05 M phosphate buffer : methanol (62 : 38 % v/v) at $P^H$ 5.9 | UV | 254 nm | 3.8 min and 5.9 min | 1.0 ml/min |
| 15. | Diazepam and Bupivacaine[15] | Enantipak (LKB) $\alpha_1$ – acid glycoprotein,(100 × 4 mm ID) | 9%, 2- propanol in 0.008 M sodium Phosphate buffer with 0.1 M sodium chloride | VWD | 215 nm | 15.7 min, 21.0 min R (+) and for s(-) enantiomer 28.3 min | 0.3 ml/min |
| 16. | Bupivacaine and Lidocaine[16] | RP – ODS Column 5 – 10µm, 300 × 4mm ID | Acetonitrile : Phosphate buffer, $P^H$6.8(65 : 35), $P^H$ 7.7 with 1M $H_3PO_4$ | UV | 263 nm | 1.0 min, 1.2 min | 2.0 ml/min |
| 17. | Bupivacaine and Lidocaine[17] | RP – ODS Column (250 × 4.6 mm) | Methanol and 1m Potassium phosphate buffer, $P^H$ 7.7 (70 : 30) | UV | 218 nm | 5.5 min, 9.5 min | 1.5 ml/min |
| 18. | Bupivacaine and tetracaine[18] | C18 Column (250 × 4.6 mm) | Acetonitrile and 11 mM triethylamine aqueous solution containing 0.1% phosphoric acid (10 : 90 v/v) and<br>Mobile phase B - Acetonitrile and 20 Mm triethylamine aqueous solution containing 0.1 % phosphoric acid (50 : 50 v/v). | UV | 210 nm | 15 min | 1.0 ml/min |
| 19. | Bupivacaine[19] | RP- C 18 column (250 × 4.6 mm) | (95 ; 5 v/v) Acetonitrile and phosphate buffer $P^H$ 3.2 | UV | 254 nm | 2 – 8 min | 1.0 ml/min |
| 20. | Bupivacaine and methylparaben[20] | RP- C 18 column (50 × 4.6 mm, 1.8 µm) | 0.02 M $P^H$ 6.8 Phosphate buffer, Acetonitrile and water in (5 : 40 : 650 v/v/v) | UV | 234 nm | 3.537 min | 1.0 ml/min |

**Table 3: High Performance liquid chromatography methods for Lidocaine**

| S.No | Drug | Stationary phase | Mobile phase | Detector | Wavelength nm | Retention timeRt (min) | Flow rate (ml/min) |
|---|---|---|---|---|---|---|---|
| 21. | Lidocaine Hydrochloride, Milrinone[21] | PXS ODS-3 Stainless steel column (250 × 4.6 mm, 10 µm particle size) | 5% acetic acid in water adjusted to [$P^H$3.0] with 1N Sodium hydroxide &acetonitrile [800:200 v/v] | UV | 254 nm | 5.0 min and 3.0 min | 2.0ml/min |
| 22. | Lidocaine Hydrochloride, and Phenylephrine hydrochloride[22] | Water µ Bondapack $C_{18}$non polar column(300 × 3.9 mm) | Aqueous solution of [54% v/v] acetonitrile containing 0.01 Monobasic potassium phosphate adjusted with $P^H$7.05 ± 0.05 with Phosphoric acid | UV | 261nm | 3.3 min | 2.0ml/min |
| 23. | Diclofenac sodium and Lidocaine Hydrochloride[23] | C18 column, (3.9 × 150 mm, 5 µm particle size) | 5% of 0.05 M orthophosphoric acid and 65% of acetonitrile | Diode array detection (DAD) | 220 nm | 5.5 and 9.5 min | 1.5 ml/min |
| 24. | Lignocaine Hydrochloride, Epinephrine Bitartarate and Atropine Sulphate[24] | Sunfire $C_{18}$ Column (250× 4.6mm, 5µm ) | Lignocaine – 50:50(v/v) of acetonitrile and water by adjusting $P^H$ 3.3 with o - Phosphoric acid.<br>Epinephrine Bitartarate – 50:50 (v/v) | PDA | Lignocaine – 254nm<br>Epinephrine | Lignocaine – 3.215 min<br>Epinephrine | Lignocaine – 1ml/min<br>Epinephrine |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | methanol and water by adjusting $P^H$ 3.2 with orthophosphoric acid. | | Bitartarate – 280 nm | Bitartarate – 3.215 min | Bitartarate – 1ml/min |
| | | | Atropine Sulphate –50:50 (v/v) methanol and water by adjusting $P^H$ 3.8with orthophosphoric acid. | | Atropine Sulphate –229 nm | Atropine Sulphate – 9.016 min | Atropine Sulphate – 0.5 ml/min |
| 25. | Lidocaine Hydrochloride, Lidocaine Hydrochloride with epinephrine Injectable solutions[25] | Water µ Bondapack CN Column (300 × 4 mm, 10 µm particle size) | 0.01 M 1- octane sulfonic acid sodium salt, 0.01 M edetate disodium, 2%(v/v) acetic acid, 2%(v/v) acetonitrile, 1%(v/v) methanol in high quality distilled water. | UV | 254 nm | 6.8 min and 3.0 min | 2.0 ml/min |
| 26. | Oxycodone and Lidocaine Hydrochloride[26] | ZORBAX SB – $C_8$ column (250 × 4.6 mm | Methanol : water : acetic acid (35 : 15 : 1 v/v/v) | UV | 285 nm and 264 nm | 10 min | 1.5 ml/min |
| 27. | Lidocaine Hydrochloride and Cetylpyridinium chloride[27] | ZORBAX (4.6mm × 250 cm column 5 µm ) | 0.05 M phosphoric acid and acetonitrile | DAD | 250nm | 3.36 min and 7.26 min | 1.2 ml/min |
| 28. | Lidocaine[28] | ZORBAX Eclipse Plus 150 × 2.1 mm, 5µm. | 50 Mm Ammonium formate: Acetonitrile $p^H$ adjusted to 8.2 | Diode array detection (DAD) | 210 nm | 4 min | 0.8ml/min |
| | | ZORBAX Eclipse Plus 50 × 2.1 mm, 3.5µm. | | | | 2 min | 0.5 ml/min |
| | | ZORBAX RRHD Eclipse Plus 50× 2.1 mm, 1µm. | | | | 0.41 min | 1.9 ml/min |
| | | Water BEH $C_{18}$, 50 × 2.1 mm, 1.7µm, | | | | 2 min | 1.5ml/min |
| 29. | Miconazole nitrate and Lidocaine hydrochloride[29] | Zorbax SB-C8 column | 0.05M phosphoric acid and acetonitrile(25: 65 v/v) | Diode array detection (DAD) | 215 nm | 4.1 to 8.4 min | 1ml/min |
| 30. | Lidocaine hydrochloride[30] | silica, 25 cm, 4.6 mm i.d., 3mm, Phenomenex, Torrance, CA. | 10 mM potassium dihydrogen phosphate buffer at pH 3.0 and methanol (50:50 v/v). | UV- VWD | 254 nm | 3.5 min | 0.85 ml/min |

**Table 4: High Performance liquid chromatography methods for Bupivacaine**

| S.no | Drug | Stationary phase | Mobile phase | Dectector | Wavelength (nm) | Retention time Rt (min) | Flow rate (ml/min) |
|---|---|---|---|---|---|---|---|
| 31. | Ropivacaine, Bupivacaine, Dexamethasone[31] | ZORBAX Eclipse XBD $c_{18}$ column (4.6 × 150 mm, 5µm particle size) | Aectonitrile-$NaH_2PO_4$ buffer 30Mm pH 3.5 adjusted with $H_3PO_4$ 30:70 v/v. | DAD | 210nm | 4.92 min | 0.8 ml/min from 0 to 7 min |
| 32. | Procaine Hcl, Bupivacaine hydrochloride, and Fentanyl citrate[32] | Lichrosphere 100 CN, 250×4mm (10 µm) column | Acetonitrile and 0.01M Phosphate buffer at $P^H$ 2.8 (3:7, V/V) with addition of 0.08 g/L of potassium chloride | UV | 210nm | 5 min, 10 min, and 15 min | 1.5 ml/min |
| 33. | Bupivacaine hydrochloride[33] | Spherisorb 5 ODS column (250 × 4.6mm; Chrompack) | 0.006 M of phosphoric acid, acetonitrile (65:35 v/v) and tetramethylammoniumchloride of 0.750 g/L. | VWD | 217 nm | 13.0 min | 1.2 ml/min |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 34. | Bisoprolol, Bupivacaine[34] | BDS Hypersil $C_{18}$ (150 mm× 4.6 mm, 5µm Thermo electron corp) | 33.5 % Acetonitrile, 66.5% of Water, 1ml/L Triethylamine, $P^H$ adjusted to 2.5 with $H_3PO_4$ | UV | 202 nm | 3.1 – 3.3 min and 3.5 -3.7 min | 1ml/min |
| 35. | Bupivacaine[35] | ZORBAX SB-CN Column (4.6mm × 1.6mm,5µm particle size) | potassium dihydrogen phosphate buffer ($NaH_2PO_4$): acetonitrile (15Mm, $P^H$7.4),(50:50 v/v) | DAD | 263nm | 5.6 min | 1ml/min |
| 36. | Pentoxifylline, Bupivacaine Hcl, Levocetrizine Hcl, Tranilast, Fluticasone Propionate[36] | Phenomenex ® Gemini (150 ×4.6 mm) $C_{18}$Guard column | Mobile phase–A: 0.02M Sodium Phosphate dibasic $P^H$ 3.3 with o-Phosphoric acid<br>Mobile phase–B : acetonitrile | UV | 220nm | 11.0-11.5 min, 13.7-14.3min, 19.7-20.4 min, 26.4-26.9 min, and 34.5-35.0 min. | 1ml/min |
| 37. | Bupivacaine[37] | $C_{18}$ column 250 mm ×4.6 mm (5µm particle size, waters Inc.) | acetonitrile and Phosphate buffer,( $P^H$3.5, 50mM) in a ratio of 70:30 v/v | UV | 210 nm | 10 min | 1.5 ml/min |
| 38. | Bupivacaine[38] | Kromasil $C_{18}$ (125 × 4.6 mm, 5 µm)<br>Prontosil $C_{18}$-AQ(125 × 4.6 mm, 5µ)<br>Luna Phenyl- Hexyl (150 × 4.6 mm, 5µ) | Methanol (Me OH)and acetonitrile (CAN) of (32: 68) (v/v). | PDA detector | 254 nm | 10 min | 1ml/min |
| 39. | Bupivacaine and Lidocaine[39] | micro Bondapak $C_{18}$, Waters Associates | Disodium hydrogen Phosphate and 0.05mol/L of acetonitrile at $p^H$ 5.8 (30:70 V/V) | UV | 210 nm | 3.5 and 4.3 min | 1ml/min |
| 40. | Bupivacaine[40] | Primesep 200 (3.2 × 100 mm) | MeCN gradient from 5% to 50 % in 5min, 4 min hold H3PO4gradient from 0.05 % to 0.3 % in 5 min, 4 min hold. | UV | 270 nm | 8 min | 1ml/min |

## CONCLUSION

Local anaesthetics are widely used to manage acute, Chronic and Cancer pain and for diagnostic purposes. They have effects in addition to preventing Sodium entry into axons that appear to contribute, at least in some instances, to their pain- reliving action. New formulations lead to prolonged action. Reverse phase HPLC and Normal phase HPLC has become important in the analysis of local anaesthetics which gives the effective results. Reverse phase and Normal phase HPLC analysis was mainly used for analysis of local anaesthetics, which gives complete characterisation of the drugs. This method also helps in studying the reaction mechanisms and also reaction pathways which helps in establishing storage conditions of the drugs.

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