Review Article

High Performance Liquid Chromatographic Methods for Local Anaesthetics – A Review

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ABSTRACT

The main goal of this review is to provide overview on the 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 normal phase HPLC in which stationary phase is polar and mobile phase is non-polar. The basic principle of HPLC includes adsorption where the component gets separated based on the relative affinities.

Keywords: Local anaesthetics, High performance Liquid Chromatographic.

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]

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 anaesthesia:**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

A. Injectable local anaesthetics	B. Surface local anaesthetics
1.Low potency, short duration local anaesthetics :-Procaine, Chloroprocaine	**a. 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(Cinchocaine)	

Table 1: 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.	**Lidocaine Hydrochloride,Milrinone[6]**	PXS ODS-3 Stainless steel column (250 × 4.6 mm, 10 µm particle size)	5% acetic acid in water adjusted to [P^H3.0] with 1N Sodium hydroxide &acetonitrile [800:200 v/v]	UV	254 nm	5.0 min and 3.0 min	2.0ml/min
2.	**Lidocaine Hydrochloride, and Phenylephrine hydrochloride[7]**	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^H7.05 ± 0.05 with Phosphoric acid	UV	261nm	3.3 min	2.0ml/min
3.	**Diclofenac sodium and Lidocaine Hydrochloride[8]**	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
4.	**Lignocaine Hydrochloride, Epinephrine Bitartarate, and Atropine Sulphate[9]**	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.	PDA	Lignocaine – 254nm	Lignocaine – 3.215 min	Lignocaine –1ml/min
			Epinephrine Bitartarate – 50:50 (v/v) methanol and water by adjusting P^H 3.2 with orthophosphoric acid		Epinephrine Bitartarate – 280 nm	Epinephrine Bitartarate – 3.215 min	Epinephrine 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
5.	**Lidocaine Hydrochloride, Lidocaine Hydrochloride with epinephrine Injectable solutions[10]**	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,	UV	254 nm	6.8 min and 3.0 min	2.0 ml/min

			1%(v/v) methanol in high quality distilled water.				
6.	**Oxycodone and Lidocaine Hydrochloride[11]**	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
7.	**Lidocaine Hydrochloride and Cetylpyridinium chloride[12]**	ZORBAX (4.6mm× 250 cm column 5 µm)	0.05 M phosphoric acid and acetonitrile	DAD	250nm	3.36 min and 7.26 min	1.2ml/min
8.	**Lidocaine [13]**	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
9.	**Miconazole nitrate and Lidocaine hydrochloride[14]**	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
10.	**Lidocaine hydrochloride[15]**	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 2: 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)
1	**Ropivacaine, Bupivacaine, Dexamethasone[16]**	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
2	**Procaine Hcl, Bupivacaine hydrochloride, and Fentanyl citrate [17]**	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
3	**Bupivacaine hydrochloride [18]**	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
4	**Bisoprolol, Bupivacaine[19]**	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
5	**Bupivacaine[20]**	ZORBAX SB-CN Column (4.6mm × 1.6mm,5µm particle size)	potassium dihydrogen phosphate buffer (NaH_2PO_4): acetonitrile (15Mm, P^H7.4),(50:50 v/v)	DAD	263nm	5.6 min	1ml/min

6	**Pentoxifylline, Bupivacaine Hcl, Levocetrizine Hcl, Tranilast, Fluticasone Propionate**[21]	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 Mobile phase–B : acetonitrile	UV	220nm	Pentoxifylline-11.0-11.5 min, Bupivacaine Hcl-13.7-14.3min, Levocetrizine Hcl-19.7-20.4 min, Tranilast-26.4-26.9 min, Fluticasone Propionate-34.5-35.0 min.	1ml/min
7	**Bupivacaine**[22]	C_{18} column 250 mm ×4.6 mm (5µm particle size, waters Inc.)	acetonitrile and Phosphate buffer,(P^H3.5, 50mM) in a ratio of 70:30 v/v	UV	210 nm	10 min	1.5 ml/min
8	**Bupivacaine**[23]	Kromasil C_{18} (125× 4.6 mm, 5 µm) Prontosil C_{18}-AQ(125× 4.6 mm, 5µ) 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
9	**Bupivacaine and Lidocaine** [24]	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
10	**Bupivacaine**[25]	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 anesthetics 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. Normal phase HPLC has become important in the analysis of local anaesthetics which gives the effective results.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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