Quality Control of Pharmaceuticals by High-Performance Liquid Chromatography

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Abstract

High-performance liquid chromatography of sixteen commonly used drugs and vitamins have been studied. Two different reverse-phase columns have been evaluated for the chromatography of drugs and related compounds. Where as non-polar and slightly polar compounds were retained quite strongly, the columns exhibited little selectivity for polar and ionic compounds. However, the retention behaviour of such compounds could be favourably influenced by variation of pH, ionic strength and organic content of the eluent. In general it was observed that the reverse-phase column is applicable to a wide range of drugs both for quality control of drugs and in situ studies (JPMA 34 35, 1984).

Introduction

High performance liquid chromatography (HPLC) is now, an established technique for analysis of drugs, for quality control, research and development work and in vivo studies such as therapeutic level in human body and study of metabolites. There are several excellent reviews published on this subject (Irwin and Scott, 1982; Gilpin, 1979; Gilpin, 1981; Brown, 1973). In fact US bureau of health and other leading world health organisations have accepted HPLC as standard technique for quality control of drugs. Although, spectrophotometery, thin layer chromatography (TLC), gas chromatography (GC), and electrophoresis are still used quite extensively for drug analysis (Gilpin, 1979; Gilpin, 1981; British Pharmacopoeia, 1973; Pharmacopoeia Francise, 1972), the HPLC methodology is gradually replacing these complementary techniques in this field. There are great advantages in this technique as it combines the separation power with quantitative and qualitative results. Comparative studies of conventional methods and HPLC methods have shown that the best results are obtained by the latter (British Pharmacopoeia, 1973; United States Pharmacopoeia, 1975). However, there are some drawbacks in using HPLC, such as the cost of equipment which may vary from ten to twenty thousands US dollars. Also a great deal of experience and expertise is required to get good results. We have analysed different commonly used drugs, which include analgesics, antibiotics, antihistamines, tranquiizers, and vitamins. The results are presented in this paper.

Experimental

Equipment

A liquid chromatographic system consisting of Beckman Model 100A solvent pump, an Altex Model 210 injection valve fitted with 20u1 loop, and a DuPont variable wavelength spectrophotometric detector was used in this study. Whenever a temperature higher than ambient was required, analyses were performed on a DuPont Model 830 liquid chromatograph fitted with a Rheodyne Model 7120 injection valve having 20 p1 loop. Either a Hewilet Packard Model 3300A, integrater/ plotter or a Schiniadzu Model CRIB laboratory data - system were used for recording chromatograms and measurement of peak areas.

Two different analytical columns were used during this study. A 4.6mm (i.d) x 250mm Zorbax ODS column was purchased from DuPont(USA), and a 4.6mm (i.d) x 250mm Partisil ODS/lO was obtained

from Whatman (Clifton, USA).

Chemicals

All solvents used in the study were of LiChrosolv grade supplied by E. Merck (Germany). Deionized water was prepared using Sybron (USA) deionizer. Other chemicals used were of analytical grade (Merck, Germany). Vitamins standards were obtained from Sigma Chemicals Co. (USA).

Sample Preparation

Accurately weighed amount of drug was dissolved in a few mis of methanol and diluted appropriately with mobile phase. In case drug contained some insoluble material the solution was first filtered through 0.45 micron millipore filter (USA) before injecting it into the column.

Results and Discussion

In reverse-phase chromatography compounds are separated on the basis of partition between two phases; the hydrophobic column packing and hydrophilic solvent. This mode of chromatography is, generally, used for the separation of non-polar or slightly polar compounds. A compound depending upon its polarity will have particular affinity for the nonpolar hydrocarbon phase of the column and also for poiar or aqueous mobile phase. The more polar or ionic solutes, which favour the aqueous eluent, elute faster in the reverse-phase HPLC.

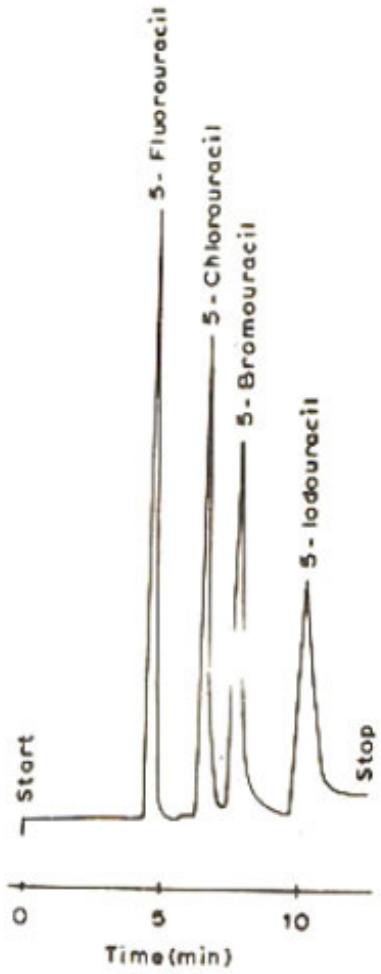

Fig. 1 Chromatogram of a mixture of halogen derivatives of uracils. Column : Partisil ODS 10/25. Mobile Phase: Water. Flow Rate: 1.0 ml/min. Detection: UV 254 nm.

Figure 1 shows a chromatogram of a mixture of different halogen substituted uracils separated on a reverse-phase column. Excellent resolution was achieved by using simply water as eluent. The fact that the similar compounds like the halogen substituted uracils can be separated by HPLC demonstrates its resolution power. Although these compounds are unlikely to be found in natural matrix, but many formulations contain active gradients which are very similar in properties and PLC is the only suitable method for the analysis of such drugs.

Compounds having greater affinity for nonpolar phas cannot be separated by purely aqueous mobile phase. Controlling the polarity of mobile phase by the addition of an organic solvent, such as methanol or acetonitril, results in displacement of molecule from the stationary phase. Separation under these conditions occur on the basis of competition or mass action effect (Kirland and Kirkland, 1967). Drugs present in ionic form are not retained on the reverse-phase column and elute with the solvent front. Retention behaviour of such species can, however, be favourably changed by adjusting the pH of the mobile phase to a suitable value. Most of the water soluble vitamins and antibiotics are ionic and therefore, affected by the pH and ionic strength of the mobile phase. Retention times and the optimum

chromatographic conditions for the analyses of different drugs studied have been summarized in table I.

Table I

Retention Time and optimum Chromatographic Conditions.

Drug	Formulation	Mobile phase	Column	Flow rate (ml/min)	Retention Time(min)	Wave Length(nm)
Aspirin	Tablet containing 300mg of aspirin.	40% KH_2PO_4 at pH 3.5, 60% Methanol.	Zipax ODS	1.0	7.52	254
Paracetamol	Tablet containing 500mg of paracetamol.	40% water, 60% methanol.	Zipax ODS	1.0	4.67	254
APC	Tablets containing Aspirin 300mg, Phenacetin and Caffeine.	40% water, 60% methanol.	Zipax ODS	1.2	2.75 (Phenacetin) 9.30 (Aspirin) 11.74 (Caffeine)	254
Novalgin	Tablets containing Sodium Phenyl-dimeth-ylamino-methane sul-phonate 500 mg.	40% water 60% methanol.		1.2	2.54	254
Frisium	Tablet containing 10mg of Clobezam.	80% 0.1M KH_2PO_4 20% acetonitrile.	Partisil ODS 10/25	1.5	8.0	254
Valium	Tablet containing 5mg of diazapam.	1)80% 0.1M KH_2PO_4, 20% acetonitrile.	1)Partisil ODS 10/25	1.5	12.9	254
		2)20% water, 80% methanol.	2)Zipax ODS	1.0	6.9	254
Ativan	Tablet containing 1mg of Lorezapam.	1)80% 0.1M KH_2PO_4, 20% acetonitrile.	1)Partisil ODS 10/25.	1.5	6.0	254
		2)30% water, 70% Methanol.	2)Zipax ODS	1.0	4.28	254
Oxytocim	Injection Oxytocyme 5 i.u/ ampule.	80% 0.1M K_2PO_4, 20% acetonitrile.	Partisil ODS 10/25	1.5	4.47	215
Penbritin	Powder, Ampicillin Trihydrate B.P. 250mg/capsule.	40% 0.1 Nah_2PO_4, 60% methanol.	Zipax ODS	1.2	3.59	254, 210 & 204,(Maxi-mum absor-ption at 204).
Septran	Tablets containing Trimethoprim B.P. 40mg Sulphamethoxazole B.P. 500 mg.	40% KH_2PO_4 (2.72g/1) at pH 3.5, 60% Methanol.	Zipax ODS	1.0	3.44 (Sulphameth-oxazole) 4.99 (Trimethoprim)	254
Chloram-phenicol	Powder, Chloromycetin 250 mg.	Methanol, water and acetic acid (55 +45+1).	Zipax ODS	1.5	7.31	278
Ampiclox	Powder, Ampicillin trihydrate B.P 250 mg and sodium colaxacillin in B.P 250 mg.	40% 0.1M NaH_2PO_4 at pH 4.0, 60% Methanol.	Zipax ODS	1.2	3.67 (Ampicillin) 6.37 (Colaxacillin)	254
5-Fluoroura-cil	Powder, 250 mg.	Water	Zipax ODS	1.0	4.74	254
Theophylline	Tablet containing 300mg of Theophline sulphate.	40% KH_2PO_4 (2.72 g/l) at 3.5, 60% methanol.	Zipax ODS	1.0	7.52	254
Phenargan	Tablet containing 25mg of Promethazine HCl.	60% methanol, 40% water.	Zipax ODS	1.0	7.05 (major peak)	254
Deltacortil	Tablets containing 1mg Prednisolone.	50% methanol, 50% water.	Zipax ODS		8.77	254

Bezodiazepines are widely used as tranquilizers. A chromatogram of a mixture of benzodiazepines

separated on an ODS column is shown in figure 2.

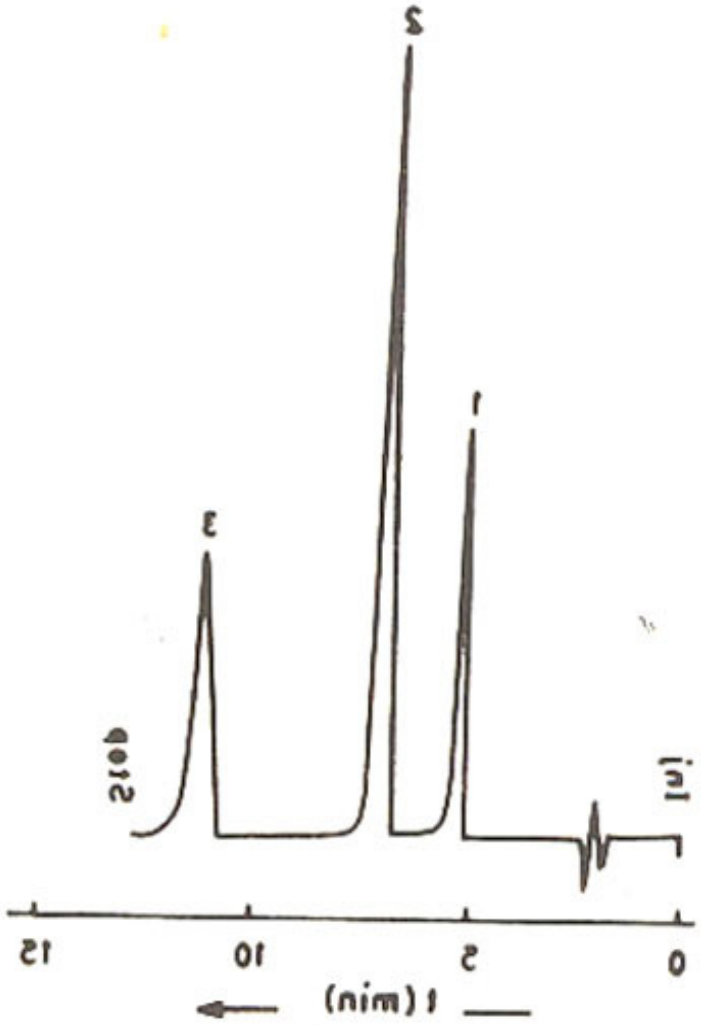

Fig. 2 Chromatogram of some Benzodiazepines. Column: Partisil ODS 10/25. Mobile Phase: 0.1M KH_2PO_4, pH 3.5 and acetonitrile (80 + 20). Flow Rate: 1.5 ml/min. Detector: 254nm. 1) Lorazapam, 2) Clobezam, 3) Diazapam.

High degree of resolution and reproducibility permits simultaneous determination of these drugs. Salicylic acid is present in aspirin as an impurity. A number of methods have been reported for its estimation in aspirin (Irwin and Scott, 1982; Menour et al., 1982). We found that best resolution was obtained by using mixture of methanol, water and acetic acid (50 + 45 + 3) as a mobile phase over a reversephase column (Figure 2). Salicylic acid upto the level of 10 ug/ml can be easily detected. Many antibiotic formulations contain more than one active components, Sometimes suiphonamides are administered in combination with other antibiotics. Simultaneous determination of these drugs is, therefore, of great analytical importance. The chromatograms of ampiclox and septran are shown in figure 3 and table II.

Fig. 3 Chromatograms of Septran and Ampiclox. Column: Zipax ODS 4.6mm (i.d.) x 250 mm. Mobile Phase: KH_2PO_4 (2.72 g/1) at pH 3.5 and methanol (40 + 60). Flow Rate 1.0ml/min. Detection 254nm.

A. Septrans–1) Sulphamethoxazole, 2) Trimethoprim.

B. Ampiclox–1) Ampicillin, 2) Colaxacillin.

Table II
Chromatographic Conditions as Described in Fig. 3.

Vitamin	Retention Time(min)
Ascorbic Acid	2.2
Pyridoxine	3.5
Niacin	4.5
Thiamine	6.5
Ribo flavin	9.2

A clean separation and reproducible results were achieved with 0.05 M KH2 P04, pH 3.5 and methanol (40 + 60) as mobile phase. Oxytocin and ampidillin do not absorb appreciably at 254nm, hence a lower wavelength setting is required to get a reasonable peak. On the other hand chioramphenicol gives a large peak at 278 nm. The use of different wavelengths suggests that a variable wavelength detector is more effective than a fixed wavelength detector.

Multivitamin formulations have no less than six or seven water soluble vitamins. These vitamins can be easily separated on a reversephase column using 0.1M KH P04 and acetonitril (84 + 16) as eluent (Figure 4).

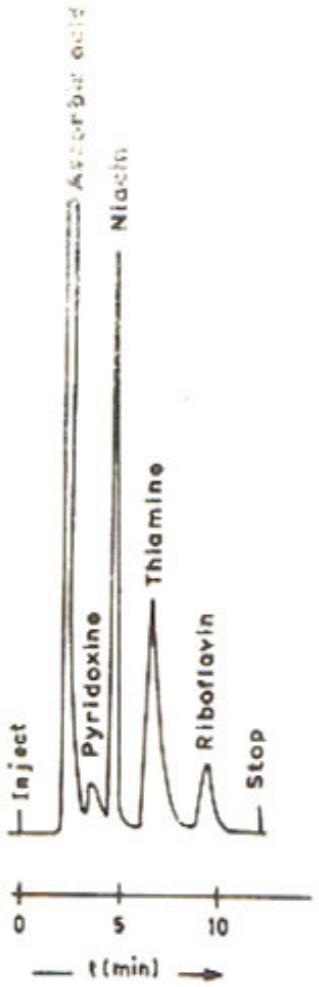

Fig. 4 Chromatogram of water soluble vitamins. Column: Partisil ODS 10/25. Mobile Phase: 0.1M NaH_2PO_4 at pH 3.5 and acetonitrile (84 + 16). Flow Rate: 1.3ml/ min. Detection: 254 nm.

Owing to the ionic structure of these vitamins their retention times are greatly influenced by the pH and ionic strength of mobile phase. Fat soluble vitamins are retained very strongly on the non-polar stationary phase, hence require a mobile phase having larger organic portion. By using gradient elution it is possible to separate all the vitamins in a single run.

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