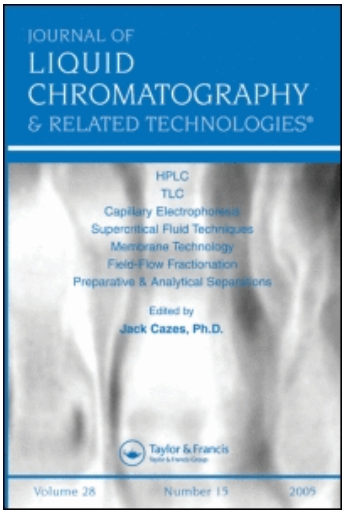

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Serum Injection of the HPLC Column for Carbamazepine Assay

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SERUM INJECTION ON THE HPLC COLUMN FOR CARBAMAZEPINE ASSAY

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ABSTRACT

Serum was injected directly on an HPLC column packed with C_1, 6.5 µm particle size, 300 Å pore-packing material for carbamazepine determination. The use of a wide-pore column with low hydrophobicity eliminated excessive pressure buildup in the column from protein precipitation which is usually caused by the high concentration of organic solvents in the mobile phase. This simple approach can be utilized for the determination of other drugs and endogenous compounds in serum.

INTRODUCTION

Carbamazepine is one of the most common drugs for treatment of complex partial seizures (1). However, the

level of this drug in serum has to be adjusted within a narrow therapeutic window (2,3). Several methods are available for assay of carbamazepine, e.g., gas chromatography, high-performance liquid chromatography and immunoassays. According to the recent therapeutic drug monitoring surveys of the American Association of Clinical immunoassays for carbamazepine, as well as for other drugs because of their speed and automation. However, immunoassays are very expensive.

Sample preparation for drugs assayed by HPLC slows the assay and adds great difficulty to this method. Direct serum injection on the HPLC column is quite desirable. It simplifies the method and helps automation; however, it results in a rapid pressure build up due to protein precipitation from the organic solvents in the column (4). Here, we describe a method which enables direct serum injection of some drugs using carbamazepine as an example. To prevent serum protein precipitation, caused by a high concentration of organic solvent, a C_1 column, which allows the use of a lower concentration of organic solvents, is used in place of the traditional C_{18} column. We also used wide-pore particles, 300 Å instead of the usual 80 Å, to keep the proteins from blocking the pores.

MATERIALS AND METHODS

Instrument: A Model 110 A pump (Beckman Instruments, Fullerton, CA) was used to pump the solvent at 1.8 mL/min. The detection was by a Model 2151 variable-wavelength detector (LKB, Broma, Sweden) set at 300 nm, 0.010 Å.

Column: A column 150 mm X 4.6 mm(i.d.) was packed with C_1, 6.5 μm average particle size, 300 Å pore size (SynChrom, Inc, Linden, IN) by the slurry-packing method in a mixture of equal volumes of isopropanol and methanol at 5000 psi at a flow rate of 10 mL/min for 40 min.

Solvent: 18% acetonitrile in water containing 100 μL/L Brij-35 (Technicon Instruments Co., Tarrytown, NY).

Method: Inject 5 μL of serum directly on the column.

RESULTS AND DISCUSSION

Small molecules traditionally are not separated on columns with wide-pore packings (protein-columns) because the large pores give lower surface area which yields a lower plate number for the columns. However, the smaller particle size, e.g., 6.5 μm, in the present column compensates, to some extent, for the decrease in plate number. The advantage of the wide-pore packing is that it has an initial low pressure that is stable after a large number of injections. Figure 1 illustrates the separation of carbamazepine in serum

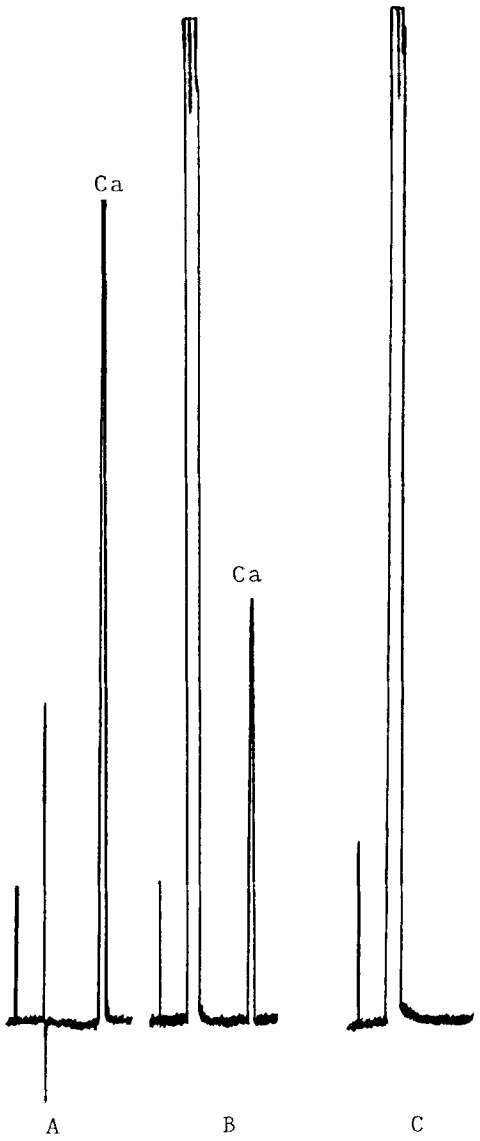

FIGURE 1: Representative chromatogram of carbamazepine (ca), of: A- standard 10 mg/L, B- serum from a patient on carbamazepine, C- serum from a patient free from the drug. (Retention time for carbamazepine peak is 200 sec.)

on the column with wide-pore packing. The present column has a plate number of 4000 based on the carbamazepine peak.

The effect of acetonitrile on the capacity factor (k') is illustrated in Fig. 2. One of the main factors in serum protein precipitation is the organic solvent concentration in the mobile phase (4). The use of C_1 packing material instead of the more popular C_{18} allowed us to reduce the acetonitrile concentration in the mobile phase from 40% to 18%. This led to a decrease in serum protein precipitation in the column. We injected about 300 samples on the column without a change in pressure. After 200 injections the peaks started to decrease in size. However, after turning the column around and washing with acetonitrile, the peak height returned to normal. Of course, the use of a guard column greatly extends the number of injections on the column.

Absorbance was set at 300 nm rather than at the maxima of carbamazepine. At this wavelength, serum proteins and endogenous compounds do not have strong absorbance, Fig. 3. Thus the chromatograms, in spite of direct serum injection, are clean. Other common drugs, such as phenytoin, primidone, phenobarbital, ethosuximide, valproic acid and theophylline did not interfere in the assay. The metabolite, carbamazepine 10, 11 epoxide, elutes earlier than the parent drug but does not have any appreciable absorbance at 300 nm.

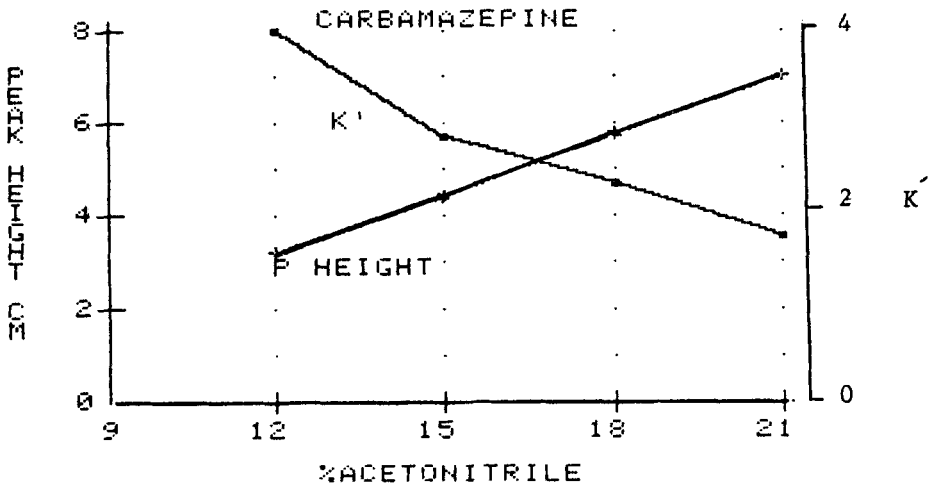

FIGURE 2: Effect of acetonitrile concentration on capacity factor (k') and peak height for carbamazepine.

We found that the addition of small amounts (100 µL/L) of Brij-35 improved slightly the reproducibility of the assay (CV = 4.8%, n = 15 in the absence and CV - 3.7%, n = 30 in the presence of Brij-35). Large amounts of Brij-35 caused proteins to elute after the carbamazepine peak. The test was linear between 2-20 mg/L. Comparison of the present method to polarized fluorescence immunoassays (TDX Abbott Laboratories, Irving, TX) yielded a good correlation between the two methods (y = 0.96 x -0.04, r = 0.97) as illustrated in Fig. 4.

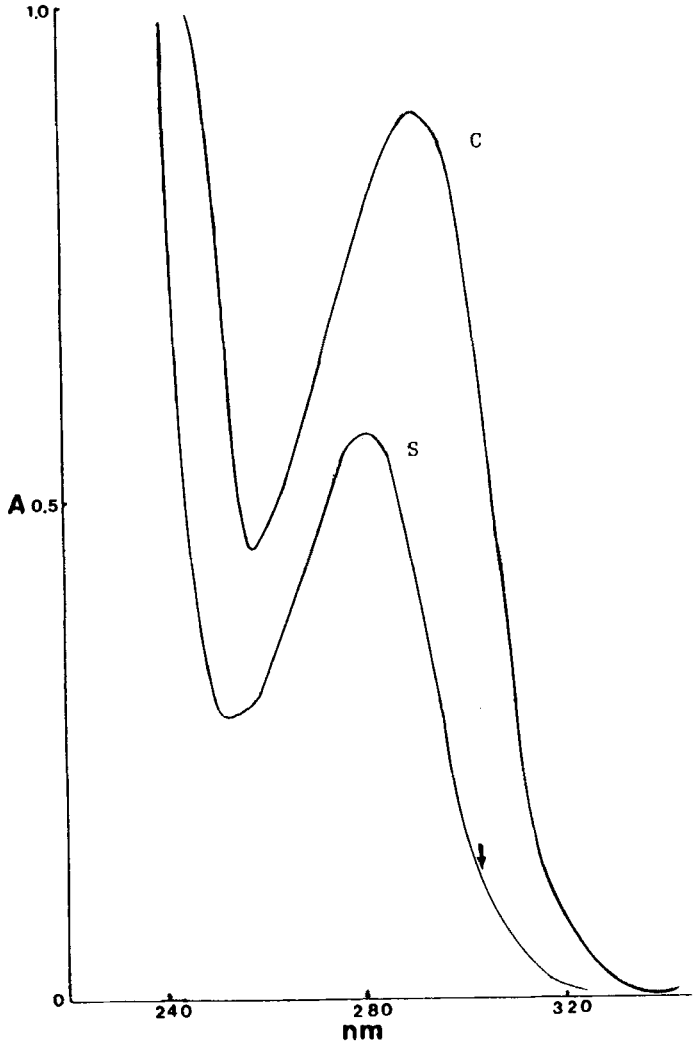

FIGURE 3: Spectra of carbamazepine (C) and serum (S) in the pump solvent.

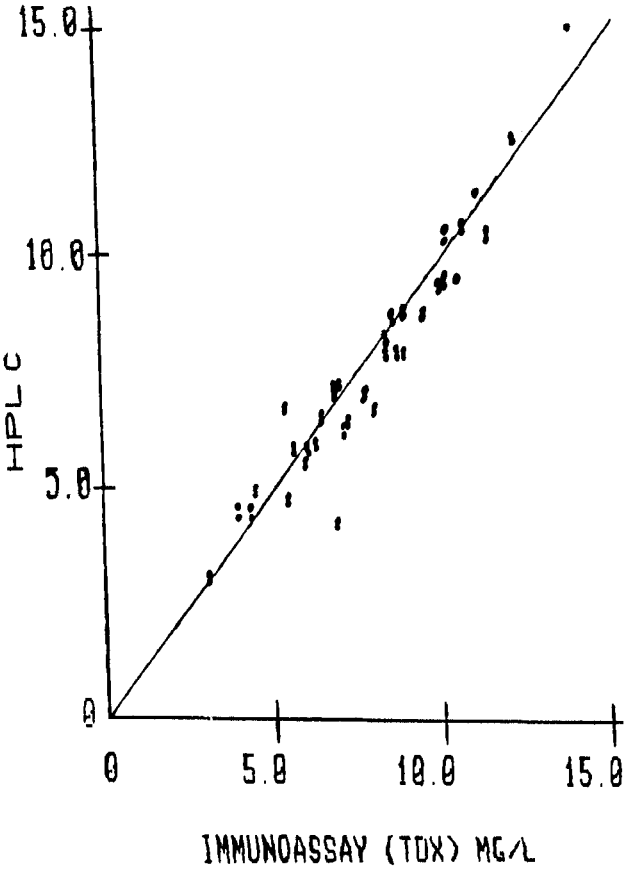

FIGURE 4: Comparison of carbamazepine determination by direct serum injection compared to polarized fluorescence immunoassay.

The present method illustrates that wide-pore packing materials, used often for proteins, give good separation for small, relatively nonpolar molecules and can be used for the determination of compounds by direct serum injections. This approach speeds up and simplifies automation of the assay. Since the packing material is relatively inexpensive, HPLC is a cost-effective alternative to immunoassays.

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REFERENCES

1. Lechtenberg R. The Diagnosis and Treatment of Epilepsy. Macmillan Publishing Co., NY, 1985, p 210.

2. Penry JK, Newmark ME. The use of antiepileptic drugs. Ann Intern Med 90, 207, 1979.

3. Bertilsson L. Clinical pharmacokinetics of carbamazepine. Clin Pharmacokin 3, 128, 1978.

4. Shihabi ZK, Dyer RD, Scaro J. Serum injection on the HPLC column for pentobarbital determination. J Liq Chromatogr. (In press.)