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## Note

# Rapid quantitative method for the simultaneous determination of carbamazepine, carbamazepine-10,11-epoxide, diphenylhydantoin, mephenytoin, phenobarbital and primidone in serum by thin-layer chromatography

## Improvement of the buffer system

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This note describes the modification of our published thin-layer chromatographic (TLC) method [1, 2] using a different buffer system. With the previously used buffer (0.3 *M* $NaH_2PO_4$) two unwanted substances from serum were extracted together with the drugs (see peaks 7 and 9 in Fig. 1). Under these conditions peak 7 can combine with the phenobarbital-peak and disappear due to small changes in the composition of the chromatographic solvent or due to unknown influences. Using a phosphate buffer almost saturated at room temperature with ammonium sulfate [500 g of $(NH_4)_2SO_4$ dissolved in 1 l of 0.3 *M* $NaH_2PO_4$; 500 µl of this buffer are added to 300 µl serum], peaks 7 and 9 disappear (Fig. 2). Since these two unnecessary substances are no longer extracted, the above-mentioned interference with phenobarbital is eliminated. The new buffer (pH = 3.9) elevates the recovery of primidone significantly (peak 1 in Figs. 1 and 2). The other drugs remain as previously reported [1].

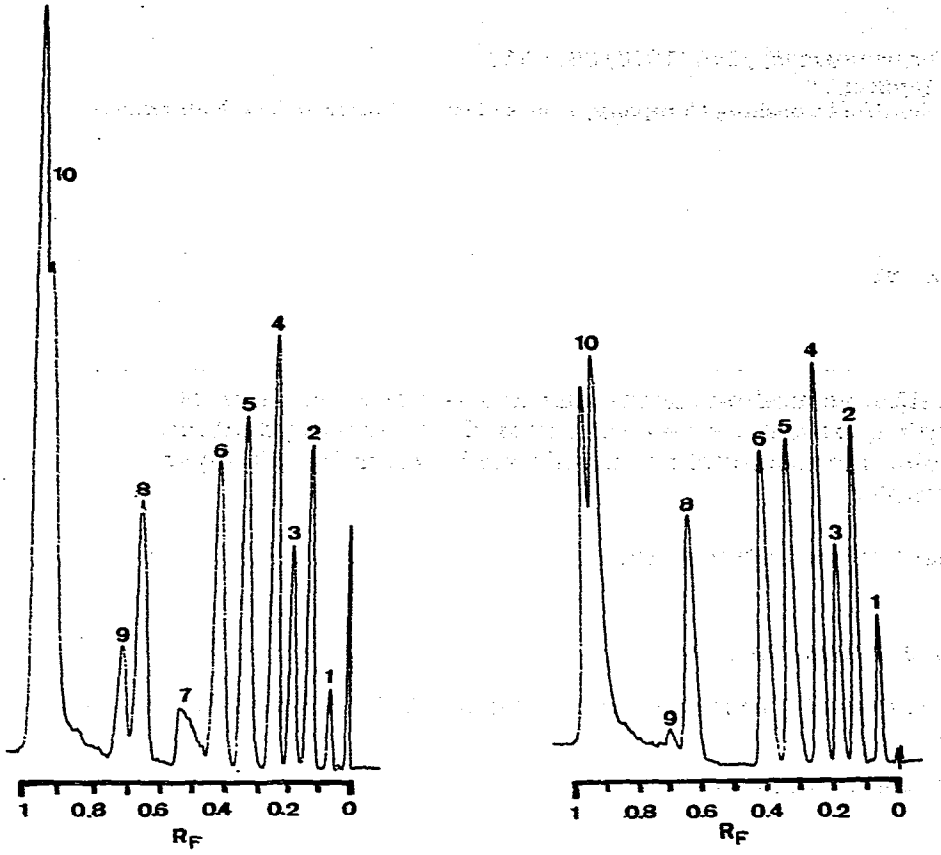


Fig. 1. Results obtained from a scan at 215 nm of a serum extract after using 0.3 *M* $NaH_2PO_4$ and TLC separation in chloroform—acetone (87:13). The serum contained 8.2 mg/l each of carbamazepine-10,11-epoxide (2), caffeine (3), carbamazepine (4) and 16.5 mg/l each of primidone (1), diphenylhydantoin (5), phenobarbital (6), mephenytoin (8). Peaks 7 and 9 are unidentified serum peaks 10 is the solvent front.

Fig. 2. Results obtained from a scan at 215 nm of an extract from the same serum and on the same TLC plate as in Fig. 1 after using 0.3 *M* $NaH_2PO_4$ which was almost saturated with $(NH_4)_2SO_4$.

## REFERENCES

1 N.T. Wad and H. Rosenmund, J. Chromatogr., 143 (1977) 89.
2 N.T. Wad and H. Rosenmund, J. Chromatogr., 146 (1978) 167.