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Simultaneous measurement of phenobarbital, carbamazepine, phenytoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin in serum by high-performance liquid chromatography.

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Abstract

A rapid, highly sensitive high-performance liquid chromatographic method has been developed for the determination of phenobarbital, carbamazepine, phenytoin and its main metabolite, 5-(4-hydroxyphenyl)-5-phenylhydantoin, in 50 μ l of serum. Serum protein was precipitated with an acetonitrile solution containing 5-(4-methylphenyl)-5-phenylhydantoin as the internal standard. The drugs were eluted from a 5 μ m, C-18 reversed-phase column at 40 °C with a mobile phase consisting of an acetonitrile-methanol-phosphate buffer of pH 4.8 (22 + 28 + 50%V/V), at a flow-rate of 1 ml min⁻¹ with UV detection at 214 nm. Each analysis required no longer than 12 min. Quantitation was achieved by the measurement of the peak-height ratio and the relative and absolute recoveries varied from 94 to 109%. Within-day coefficients of variation ranged from 1.2 to 3.22% and between-day coefficients of variation from 2.0 to 3.4% in subtherapeutic, therapeutic and toxic concentrations.