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Simultaneous determination of newer anti-epileptic drugs (AEDs) by liquid chromatography mass spectrometry (LC-MS)

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AIMS: To present a simple and reliable LC-MS method for the simultaneous determination of newer anti-epileptic drugs (gabapentin, levetiracetam, topiramate and vigabatrin) in serum for use in clinical toxicology.

METHODS: Aliquots (200µl) of serum were deproteinised with 1 ml of acetonitrile containing acetaminophen as internal standard. The supernatant (300µl) was removed, dried under nitrogen gas at 50°C and the dried extracts were reconstituted with 200µl of mobile phase. Analysis was carried out on an Agilent LCMS system with electrospray ionization (ESI) in both the positive and negative ionization mode. The compounds are identified based on the ion ratios of the respective target and qualifier ions. The AEDs were chromatographed on a Hypersil-BDS column (2.1 mm i.d. × 15 cm, 5 µm particle-size) with gradient elution using mobile phase containing 2 mM ammonium formate (pH ~3) and acetonitrile.
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RESULTS: The present method was fully validated and linear calibration curves (with correlation coefficients greater than 0.99) were obtained in the concentration ranges from 2.5 to 20 µg/ml for levetiracetam and from 2.5 to 40 µg/ml for the other three AEDs (gabapentin, topiramate and vigabatrin). The intra- and inter-day coefficients of variation at three different concentrations were both <10%. Limits of detection for all four AEDs were at least 0.5 µg/ml and the limits of quantification were 1.0 µg/ml. Three other drugs used in anti-epileptic therapy (phenytoin, carbamazepine and phenobarbitone) and forty-three commonly prescribed drugs were tested for possible interference. No interference was observed at the retention times of all four AEDs monitored. The absolute recoveries for the four AEDs were found to be in the range of 22% to 35%, and the relative recoveries were found to be in the range of 54% to 81%.

CONCLUSION: The presented method is simple, selective and provides adequate sensitivity for simultaneous screening and quantification of all four anti-epileptic drugs. The method has also been successfully applied to inter-laboratory quality assurance samples.

KEYWORDS: *Gabapentin, Levetiracetam, Topiramate, Vigabatrin, LC/MS*

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