Research article

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# A rapid and simple assay for lamotrigine in serum/plasma by HPLC, and comparison with an immunoassay

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**ABSTRACT: Monitoring serum/plasma concentrations of lamotrigine may be useful under certain circumstances. An HPLC column packed with strong cation-exchange (SCX)-modified microparticulate silica together with a 100% methanol eluent containing an ionic modifier permits direct injection of sample extracts. An HPLC-UV method developed using this principle for the measurement of serum/plasma lamotrigine is simple, sensitive and selective. The analysis time is less than 5 min. Intra- and inter-assay precision and accuracy meet acceptance criteria, and sample stability, and potential interferences from other compounds have been evaluated. There was good agreement with consensus mean results from external quality assessment samples ($n = 32$). Analysis of patient samples ($n = 115$) using the HPLC method and the Seradyn QMS® Lamotrigine immunoassay showed that the immunoassay over-estimated lamotrigine by 21% on average. Copyright © 2010 John Wiley & Sons, Ltd.**

**Keywords:** lamotrigine; strong cation-exchange HPLC; non-aqueous ionic eluents; immunoassay

## Introduction

Lamotrigine (Fig. 1) is used in the treatment of epilepsy, either alone or in combination with other anticonvulsants. It is also used as a mood stabilizer in bipolar disorder, for example. Lamotrigine dose adjustment is not straightforward due to (i) large inter-patient variations in dose requirement and (ii) important interactions with other anticonvulsants, notably phenobarbital, carbamazepine, phenytoin and valproic acid. The measurement of serum or plasma lamotrigine can therefore be useful, particularly during dose adjustment and when combination anticonvulsant therapy is initiated or withdrawn (Angelis-Stoforidis *et al*., 1999; Johannessen *et al*., 2003; Subramanian *et al*., 2008).

Published methods for serum/plasma lamotrigine measurement are generally based on reversed-phase HPLC (RPLC) with UV detection (Angelis-Stoforidis *et al*., 1999; Barbosa and Mídio, 2000; Beck *et al*., 2006; Saracino *et al*., 2007; Greiner-Sosanko *et al*., 2007), usually involving evaporation of sample extracts followed by reconstitution in eluent prior to chromatographic analysis. The strong cation-exchange (SCX) HPLC-UV method developed allows direct injection of sample extracts and is simple, fast, sensitive and selective. Method validation incorporated a comparison with the results obtained from patient samples using the Seradyn QMS® lamotrigine immunoassay.

## Experimental

### Chemicals and reagents

Lamotrigine was a gift from GlaxoSmithKline (Stevenage, UK). Sodium hydroxide (reagent grade), tris(hydroxymethyl)aminomethane (Tris, ACS grade) and nortriptyline hydrochloride (min 98%) were from Sigma (Poole, UK). Ammonium perchlorate (≥98%) and perchloric acid (60%) were from Fluka (Poole, UK). Methanol and methyl *tert*-butyl ether (MTBE; both HPLC grade) were from Rathburn (Walkerburn, UK), and NIST-traceable pH reference buffer solutions were from Merck (Beeston, UK). Coarsely filtered pooled human serum was from Scipac (Sittingbourne, UK).

Tris solution (2 mol/L, pH 10.6) was prepared by dissolving 232 g Tris in deionized water (total volume 2 L), and the pH adjusted using aqueous hydrochloric acid (6 mol/L). Internal standard solution (5 mg/L nortriptyline free-base) was prepared by dilution of a stock solution (500 mg/L free-base in 0.1 mol/L hydrochloric acid) with deionized water, and stored at 2–8°C.

A stock HPLC eluent solution (100 mmol/L) was prepared by adding 23.5 g ammonium perchlorate to a 2 L volumetric flask containing 20 mL of sodium hydroxide (0.1 mol/L) in methanol, and making up to volume with methanol. This solution was diluted with methanol to give an eluent ionic strength of 35 mmol/L ammonium perchlorate. The eluent was vacuum filtered (0.45 µm, nylon 66) and the apparent pH (pH*) adjusted to 6.0 using perchloric acid (1% v/v in methanol) before use. The combination pH electrode (Liq-Glass; Hamilton) was calibrated using aqueous buffers (pH 4.00, 7.00, and 10.00).

### Materials and equipment

Glass test tubes (60 × 7 mm, Dreyer tubes) were from Esslab (Benfleet, UK). Sample/calibration solution/internal quality control (IQC) solutions were pipetted using variable volume air-displacement pipettes (BioHit, Finland), and internal standard added using a repeating volume dispensing pipette (Multipette®, Hamilton). Extended fine-tipped pastettes were

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**Abbreviations used:** EQA, external quality assessment; IQC, internal quality control; MTBE, methyl *tert*-butyl ether; SCX, strong cation-exchange.

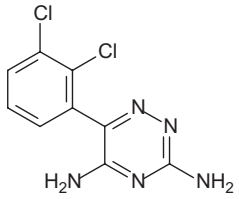


**Figure 1.** Structural formula of lamotrigine.

used to transfer sample extracts into 0.5 mL polypropylene micro tubes with lids (Sarstedt, UK). Other equipment included vortex mixers, 2 mL polypropylene screw-top tubes and a micro-centrifuge.

The HPLC system consisted of a pump (3185PU), autosampler (3059AS), UV–vis detector (3075UV, 220 nm) and column oven (CO2067; all Jasco, Great Dunmow, UK). The autosampler tray was maintained at 10°C. Data acquisition and processing were performed using EZChrom Elite (Agilent, version 3.2.1). A stainless-steel HPLC column (100 × 2.1 mm i.d.) packed with Waters Spherisorb S5SCX (propylsulfonic acid-modified) silica (HiChrom, UK) was used with a stainless-steel guard column (10 × 2.1 mm i.d.) packed with the same material. The column oven temperature was maintained at 35°C. The eluent flow-rate was 0.5 mL/min, and the injection volume was 40 µL. Difference plots and *t*-test results were produced using Analyse-it (version 2.21) within Microsoft Excel.

### Preparation of calibration and internal quality control solutions

Stock solutions (1.00 g/L lamotrigine) were prepared by weighing 50.0 mg lamotrigine into volumetric flasks (50 mL) and making up to volume with hydrochloric acid (0.1 mol/L). Calibration solutions were prepared by dilution of a stock solution with pooled human serum to give solutions containing 0.5, 2.0, 5.0, 10.0, 20.0 and 30.0 mg/L lamotrigine. IQC solutions containing 1.0, 8.0 and 25.0 mg/L lamotrigine were similarly prepared in pooled human serum from a separate stock solution. After thorough mixing and equilibration (24 h, 2–8°C), the solutions were divided into approximately 200 µL portions and stored in 2 mL polypropylene tubes at −20°C until required.

### Sample preparation

Sample, calibration solution or IQC solution (50 µL), internal standard (50 µL) and Tris solution (50 µL) were vortex-mixed (5 s) in a Dreyer tube. MTBE (200 µL) was added, and the tube contents vortex-mixed (20 s) and centrifuged (11,000***g***, 4 min). A portion (>60 µL) of the MTBE extract (upper layer) was added to a micro tube, and the tube was capped.

### HPLC method validation

The peak area ratio of lamotrigine to the internal standard was plotted against concentration of lamotrigine in the calibration standards, and a line fitted by least squares regression. Calibration standards and IQC solutions were assayed at the beginning and end of each analytical sequence, with IQCs also repeated after every 10 injections. Patient samples were analysed in duplicate. Assay acceptance criteria were (i) correlation coefficient >0.98, and (ii) IQC values within ±15% of their nominal value. Intra- and inter-assay precision (% RSD) and accuracy were measured by replicate analysis ($n$ = 10) of the IQC solutions on the same day, and duplicate analyses (mean of duplicates) on different days ($n$ = 5), respectively. The stability of lamotrigine in serum was evaluated by analysis of IQC samples (i) through three freeze–thaw cycles, the assay being calibrated each time with standard solutions that had not been thawed previously, and (ii) before and after standing for 24 h at room temperature. Recovery of lamotrigine was investigated by comparison of the mean peak area of lamotrigine from extracted solutions of each level of IQC ($n$ = 3) with the mean peak area obtained from non-extracted solutions at the equivalent concentration ($n$ = 10). Serum samples ($n$ = 32) containing lamotrigine from the UKNEQAS Heathcontrol TDM external quality assessment (EQA) scheme (Cardiff Bioanalytical Services, UK) were assayed retrospectively, but without prior knowledge of the consensus mean results.

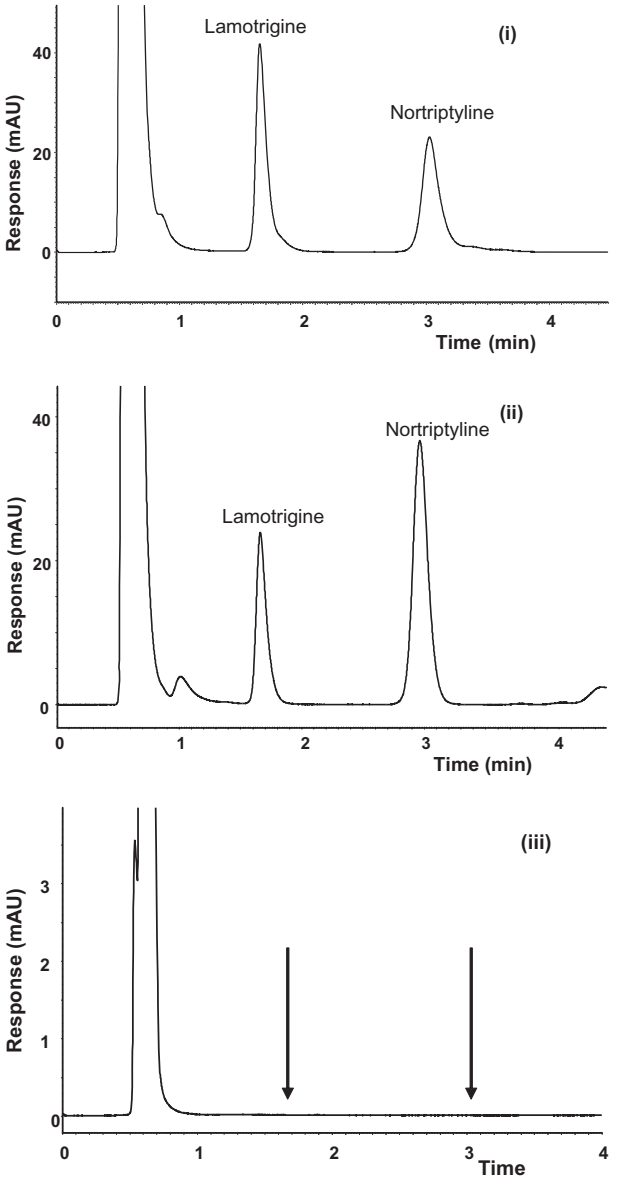


**Figure 2.** Chromatograms of serum extracts from (i) internal quality control solution (8 mg/L lamotrigine), (ii) patient sample (prescribed dose of lamotrigine 200 mg/day, concentration of lamotrigine 4.1 mg/L), and (iii) analyte-free serum without addition of internal standard (arrows indicate the retention times of lamotrigine and nortriptyline).

## Results

Typical chromatograms are shown in Fig. 2. The calibration graph was linear over the range used. Injection (2 µL) of separate solutions containing leviteracetam, oxcarbazepine, vigabatrin and rufinamide (all 10 mg/L in methanol) showed that these compounds were unretained. Furthermore, the presence of other

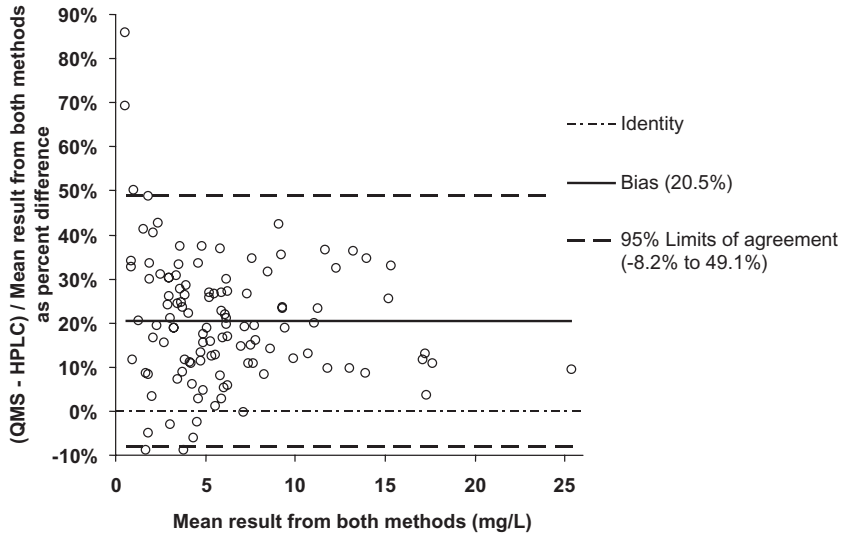


**Figure 3.** Difference plot: QMS® Lamotrigine assay and HPLC assay, samples from patients prescribed lamotrigine ($n = 115$).

anticonvulsants in the EQA material did not interfere with either lamotrigine or nortriptyline. Out of a range of psychoactive compounds also tested under these conditions, desipramine, norclomipramine and protriptyline may interfere with nortriptyline, and fluvoxamine with lamotrigine.

Inter- and intra-assay precisions (RSD) were in the ranges 3.1–4.1 and 4.2–4.4%, respectively, with accuracy between 101 and 103% of nominal concentrations. Recovery of lamotrigine from human serum was 81 ± 3%. No significant loss of lamotrigine from serum was observed during the stability studies. Results from the re-analysis of calibration standards and IQC solutions after storage for 14 months at –20°C (assay calibrated using standard solutions prepared from freshly prepared stock solutions) gave measured lamotrigine concentrations within ±16% of nominal. The limit of accurate measurement, taken as a peak of 5 times the average height of the standard deviation of the baseline noise, was 0.1 mg/L.

EQA samples were assayed in duplicate. Samples that did not contain lamotrigine ($n = 3$) were identified as such by the HPLC method. Results ($n = 29$, excluding three not containing lamotrigine) showed good agreement with consensus mean results. Comparison using linear regression gave: slope (95% CI) = 1.03 (1.01–1.05); *y*-axis intercept (95% CI) = 0.04 (–0.06 to 0.14); $R^2 = 0.998$.

### Comparison with immunoassay

The HPLC method described was used to compare results from serum samples from an EQA scheme (UKNEQAS Heathcontrol TDM, $n = 32$) and from patients prescribed lamotrigine ($n = 115$) with the Seradyn QMS® immunoassay. No significant difference was observed for the EQA samples (paired *t*-test, $t = –0.51$). However, for the patient serum samples the immunoassay results were some 20.5 ± 1.36% higher than those obtained by HPLC (paired *t*-test, $t = –11.45$, d.f. = 114, $p = <0.001$; Fig. 3). Measurement of the serum calibrators provided with the immunoassay kit by HPLC gave the following results (stated/found concentration, mg/L): 2.0/2.4; 5.0/5.0; 10.0/9.4; 20.0/19.2; 40.0/38.9.

## Discussion

A previous study (Westley and Morris, 2008) reported a significant overestimation (6%) of lamotrigine concentrations in patient samples measured using the Seradyn QMS® immunoassay compared with HPLC. In our study, this bias was 21%. While Westley and Morris used plasma, our patient samples were serum, which may account for some of the difference. However, the manufacturer states that the QMS® Lamotrigine assay is suitable for either serum or plasma. The observation that the QMS® immunoassay showed no significant bias with the EQA samples compared with the HPLC method developed suggests that there may be cross reaction with lamotrigine metabolites or other components present in patient samples, and that further investigation may be necessary.

The advantages of using SCX-modified microparticulate silica HPLC packings in basic drug analyses have been documented (Flanagan *et al*., 2001; Morgan *et al*., 2010). Despite being a weak base ($pK_a$ 5.5–5.7), lamotrigine is retained on the SCX column under the conditions described. The inherent selectivity of SCX for protonated analytes results in no interference from other anticonvulsants, offering a real alternative for lamotrigine assay. On the other hand, this method is not suitable if other anticonvulsants need to be measured at the same time as lamotrigine. The potential for interference from desipramine, norclomipramine, protriptyline and fluvoxamine, and indeed from nortriptyline, is very low in practice even if co-prescribed with lamotrigine, since the concentrations of these compounds achieved in therapy are relatively low.

## Conclusions

We have developed and validated a fast, straightforward HPLC method for serum/plasma lamotrigine measurement. The method is selective, accurate and allows direct injection of sample extracts. Comparison of results from patient samples with those from the Seradyn QMS® Lamotrigine immunoassay indicated that the immunoassay should be used with caution until further evaluations/investigations have been completed.

## Acknowledgements

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