Multiplex Mass Spectrometry Analysis of Latest-Generation Antiepileptic Drugs: A Clinically Useful Laboratory Tool for Improved Real-Time Patients' Care

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Summary

This article is a brief overview of the recent developments in clinical laboratories opening the way to a facilitated access to real-time Therapeutic Drug Monitoring (TDM) of newer antiepileptic drugs (AEDs). New highly sensitive and selective methods by mass spectrometry make it now possible to analyze arrays of several structurally unrelated AEDs simultaneously. Drug-levels quantification and expert TDM interpretation can be available within 6-24 hours, therefore improving real-time patients' care. Further analytical developments may include the measurement of AEDs in saliva, improving patients' convenience. In the future, TDM might involve miniaturized automates for point-of-care testing in analogy with glucose measurements in patients with diabetes.

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Keywords: Therapeutic drug monitoring, effectiveness, AEDs

Analyse durch Multiplex Massenspektrometrie bei Antiepileptika der letzten Generation: ein klinisch interessantes Werkzeug für ein verbessertes Patientenmanagement

Dieser Artikel stellt eine Übersicht der neuen Entwicklungen der Laboranalysen dar, welche eine vereinfachte Messung der Plasmaspiegel von den neuen Antiepileptika erlauben. Hochsensitive Leistungen durch Massenspektrometrie können eine rasche Spiegelmessung von strukturell unterschiedlichen Substanzen in der gleichen Zeit gewährleisten; dies führt dazu, dass Resultate innerhalb von 6 - 24h zu Verfügung stehen, mit einer signifikanten Besserung des Patientenmanagements. Eine weitere mögliche Entwicklung betrifft die Benutzung des Speichels anstatt des Blutes als Substrat, mit einem erhöhten Komfort für die Patienten. Schliesslich, sind in der Zukunft miniaturisierte Automaten vorstellbar, die Messungen in unmittelbarer Nähe der Patienten erlauben, in Analogie mit den Glukometern bei diabetischen Patienten.

Schlüsselwörter: Therapeutisches Monitoring, Effektivität, Antiepileptika

Analyses en multiplex des nouveaux médicaments antiépileptiques par spectrométrie de masse : un nouvel outil de laboratoire pour une meilleure prise en charge des patients en temps réel

Cet article est une revue des récents développements réalisés au laboratoire pour l'analyse par spectrométrie de masse des nouveaux médicaments antiépileptiques. L'application de cette technologie permet d'avoir un accès facilité aux mesures de concentrations plasmatiques de médicaments. La sensibilité et la sélectivité des méthodes par spectrométrie de masse en tandem permettent de quantifier en temps réel plusieurs médicaments antiépileptiques simultanément. Les résultats analytiques et leurs interprétations TDM par des experts en pharmacologie clinique sont disponibles dans les 6 à 24 h, ce qui permet d'améliorer la qualité de la prise en charge des patients. Les développements futurs s'orientent sur la mesure des taux d'antiépileptiques dans la salive ce qui va également faciliter le suivi des patients. Dans le futur, il est aussi envisageable de disposer d'automates miniaturisés permettant la mesure des taux d'antiépileptiques au chevet du patient, par analogie à ce qui se fait déjà avec la mesure de la glycémie chez les patients diabétiques.

Mots clés : Suivi Thérapeutique des Médicaments, efficacité, antiépileptiques

Analisi dei nuovi medicamenti antiepilettici per spettrometria di massa multipla: un approccio clinicamente interessante per una migliore gestione dei pazienti

Questo articolo passa in rassegna in modo mirato gli sviluppi recenti concernenti le analisi di laboratorio tese a facilitare il monitoraggio in tempo reale dei tassi plasmatici dei nuovi medicamenti antiepilettici. La spettrometria di massa ad alta sensibilità permette l'analisi contemporanea di diverse sostanze senza relazione strutturale reciproca. La quantificazione delle concentrazioni e l'intepretazione farmacologica sono così disponibili nelle 6 - 24 ore, con un indubbio impatto favorevole sulla gestione del paziente. Gli sviluppi futuri dovrebbero permettere l'analisi della saliva al posto del sangue, nonché la possibilità d'utilizzare apparecchi miniaturizzati per un monitoraggio "al letto del malato", in analogia con i glucometri diffusi da anni presso i pazienti con diabete.

Parole chiave: Monitoring terapeutico, effettività, antiepilettici.

Therapeutic Drug Monitoring (TDM) for antiepileptic drugs (AEDs)

It has been established over the last decades that the therapeutic use of AEDs could be optimized by an individualization of their dosage, based on blood (plasma) concentrations measurement. This is done via Therapeutic Drug Monitoring (TDM), which represents current practice for the "classical" antiepileptic drugs, namely phenytoin, phenobarbital, carbamazepine and valproate, all of them being characterized by relatively narrow therapeutic indexes and significant inter-individual pharmacokinetic variability. These last years, a large number of "newer" AEDs, belonging to various unrelated chemical classes, have emerged [1], and additional agents are at a late stage of development. This continuously growing armamentarium constitutes a formidable wealth of new therapeutic options for patients with epilepsy. While these last generation drugs are characterized by more favorable safety and tolerability profiles as compared to "classical" AEDs, a number of issues remain, including unpredictable pharmacokinetics influenced notably by patients' patho-physiological conditions, such as renal or hepatic function fluctuations or alterations, especially in special patient sub-groups (elderly, pregnancy, etc).

If TDM is considered for the efficient clinical use of the latest-generation AEDs, information on plasma levels must be available to the treating clinician within a few hours for dose adjustment. Rapid, sensitive and selective laboratory methods are therefore needed for an efficient TDM.

New powerful clinical laboratory methods

A comprehensive review has been recently published on the advances of both commercial and laboratory-developed AEDs testing [1]. Over the last 30 years, plasma levels of the most common AEDs, especially the "classical" generation, have been measured in clinical laboratories by gas chromatography [2] or by high performance liquid chromatography (HPLC) [3-5], as well as by immunoassays [6-8]. However, immunoassays and HPLC methods that have been mostly used so far have several recognized limitations. Immunoassays are known not to be specific to the parent drug only but also capture the signal of structurally related metabolites. HPLC methods with UV detection are probably more specific but still remain vulnerable to components that may co-elute chromatographically with the target analyte. Increased selectivity may be achieved by slowing the chromatographic gradient program, resulting however in prolonged analytical times and Turn-Around-Times (TAT). In addition, immunoassays are restricted to the measurement of one single drug at a time.

All these limitations have been circumvented by the recent development of high- or ultra-performance Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) that qualifies for the rapid, highly specific analysis of arrays of structurally unrelated AEDs simultaneously. However, it is only recently that this technological advance has been formally exploited for the TDM of latest-generation AEDs. Recent assays imply most generally the measurement by LC-MS/MS of a single drug (including, but not restricted to, lacosamide [9]; lamotrigine [10, 11]; zonisamide [12]; levetiracetam [13] and its metabolite [14]; topiramate [15-17]) determined in plasma, and for some of them, in the saliva [18] and even in dried blood spots [19]. Recently, assays by HPLC or UPLC-MS/MS have been proposed for the determination of several compounds simultaneously [20-22], even up to as many as 22 AEDs (including "classical" and "newer" ones) [23]. However, this method used only a limited number of [21] or a single [22] or no [20, 23] stable isotopically-labelled internal standards that are needed to fully compensate for the potential deleterious influence of plasma matrix variability. Since highly variable plasma or serum matrices do occur in special conditions, such as pregnancy and pediatric patients, or in the heterogeneous patients' populations in the ICU or emergency settings, this point is relevant. These patients are frequently polymedicated and/or characterized by significant alteration of renal and hepatic function that likely affects blood and plasma matrix composition.

Last year an assay for six AEDs by UPLC-MS/MS was published, which included the use of stable, isotopically labeled internal standards for all drugs [24]. This landmark publication was the first report of a comprehensively validated assay for the most frequently used

last generation AEDs. In general, short analytical runs, typically lasting less than 10 minutes, are required to provide clinicians in a timely manner with plasma levels results and TDM interpretations.

A comprehensive analytical service for real time TDM of last generation AEDs

Following a request arising from our epileptologists, we have developed in our laboratory an ultra-performance liquid chromatography-tandem mass spectrometry method (UPLC-MS/MS) requiring as low as 100 µL of plasma (or serum) for simultaneous quantification within 7 min of the five frequently used AEDs, namely levetiracetam, zonisamide, lacosamide, lamotrigine and topiramate.

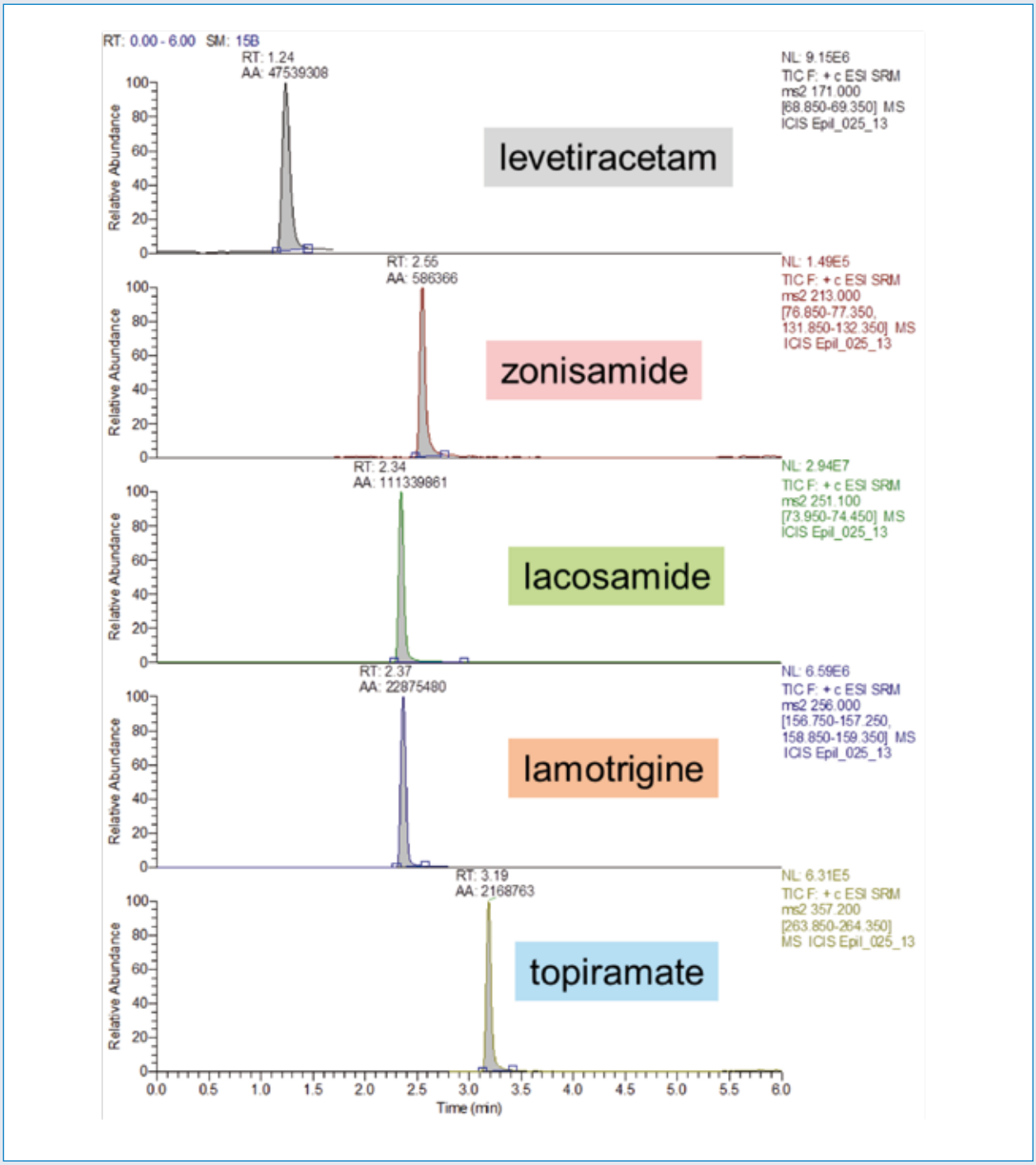

Figure 1: Multiplex analysis by ultra-performance liquid chromatography-tandem mass spectrometry method (UPLC-MS/MS) of "newer" antiepileptic agents. Chromatographic profiles of a plasma calibration sample at 10 µg/ml.

An example of the chromatographic profile of our multiplex assay is shown in **Figure 1**. Each AED is monitored using its specific *m/z* mass spectrometry transition (i.e. the chemical footprint of the molecule). When the drug is eluted from the chromatographic column, a signal presents as a peak, whose intensity is a function of the concentration in the plasma. Each drug signal is normalized with its corresponding stable, isotopically-labelled internal standards added to the patient's sample for correcting potential matrix effects (i.e. levetiracetam-d6, zonisamide-d4,^{15}N, lacosamide-^{13}C,d3, lamotrigine-^{13}C7^{15}N, and topiramate-d12 (not shown in **Figure 1**). The calibration is established within the clinically relevant range of concentration, generally comprised between 0.1 µg/ml (limit of quantification) and 50 µg/ml. This multiplex assay using a simplified extraction procedure followed by simultaneous quantification of multiple AEDs is highly efficient for rapidly providing TDM results, allowing real-time processing of blood samples from patients receiving either different single-drug or combined regimens. Of note, we participate in an external quality program for AEDs where our laboratory performs very well (100 % correct results in the last 4 rounds).

One of the major advantages of mass spectrometry assays is their flexibility to adapt them, if necessary also integrating the analysis of newer drugs (e.g., perampanel). Thus, the list of agents included in the multiplex assay is likely to evolve according to the new therapeutic options and recommendations that may emerge in the future.

Practical considerations

Fortunately, the current five AEDs included in our assay have been shown to be stable for at least 3 days in whole blood at +4°C and at room temperature [24], which is particularly convenient when considering sending samples by post throughout Switzerland, as delivery time to the laboratory for fast post (*"courrier A"*) is 24 - 48h (opening days). Blood samples (2.7 ml) containing citrate as anticoagulant is preferred (for example, in Monovettes®, Sarstedt, Nümbrecht, Germany), but serum or plasma EDTA are also acceptable. It is recommended to send the whole blood samples by post in protective plastic tubes for minimizing the risk of blood spilling. Besides, the blood sample needs to be accompanied with all information required for an expert TDM interpretation: this information has to be carefully recorded in dedicated TDM request forms, an example thereof can be downloaded from the Division of Clinical Pharmacology website [25].

Perspective

Less invasive methods for "newer" AEDs monitoring are needed to improve clinical care and patients' convenience, especially in an emergency and ICU environment, but also in an out-patient setting. The drug determination in saliva (i.e. oral fluid) that reflects the free (unbound, pharmacologically active) concentration in plasma constitutes an attractive option, but requires the high sensitivity provided by UPLC-MS/MS technology notably for drugs highly bound to circulating plasma proteins (i.e. with low free fractions). Nevertheless, before saliva can be considered as a suitable fluid for TDM implementation on large-scale, the strength of the correlation between saliva and free and total plasma levels should be evaluated; this is the subject of an ongoing prospective observational study in our Hospital.

In the future, the current reliance of TDM on large central laboratories equipped with costly LC-MS/MS apparatus will possibly be challenged by the development of miniaturized devices designed for point-of-care testing. Innovative « lab-on-chip » technologies, coupled with intelligent computer-assisted interpretation of concentration results, might indeed revolutionize the practice of TDM and contribute to extend it largely to everyday patients' care. It is highly conceivable that this approach will be applied to blood as well as saliva samples, provided its accuracy, precision and clinical usefulness have been rigorously established. We are currently participating in research efforts aimed at establishing such a concept [26].

Conclusion

This robust, sensitive and selective multiplex UPLC-MS/MS method allows the accurate and precise quantification of plasma concentrations of five of the most frequently used "newer" AEDs simultaneously, with high throughput, i.e. with a single plasma extraction and analytical runs lasting a few minutes. This new method is characterized by an excellent extraction yield and by the use of isotopically-labeled internal standards that can confidently compensate for any matrix effect variability. This approach allows providing TDM service with rapid turn-around time. The developed assay is also flexible, being able to evolve by also including, after slight adjustments, any new drugs for the treatment and prevention of epilepsy. This new method providing analytical results within 6 to 24h offers an efficient tool for a tailored drug dosing and optimization of efficacy and safety in patients with epilepsy.

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