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Chemometric approach to open validation protocols
Prediction of validation parameters in multi-residue ultra-high performance liquid chromatography–tandem mass spectrometry methods

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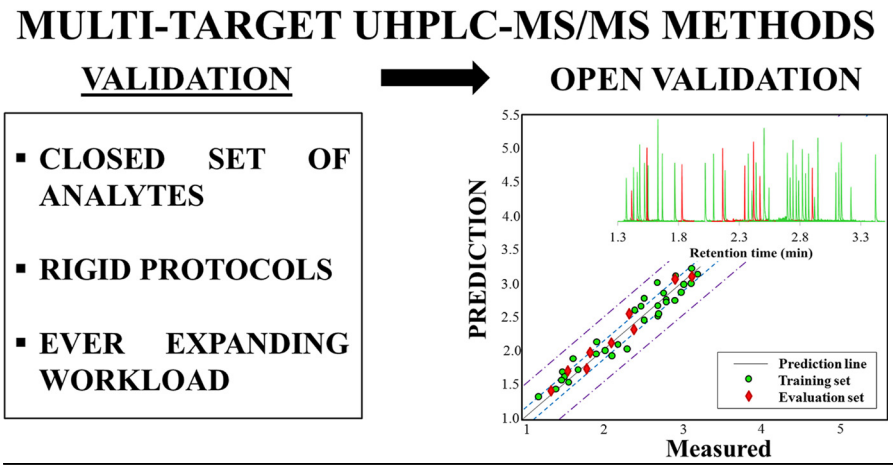
HIGHLIGHTS

- Prediction of UHPLC-MS/MS method validation parameters is made possible by chemometrics.
- Partial least squares regression computation is used to make prediction on validation features.
- Genetic algorithms are used to select the model influential variables.
- Prediction by chemometric tools opens new opportunities in the development of open validation protocols.

GRAPHICAL ABSTRACT



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ABSTRACT

The recent technological advancements of liquid chromatography–tandem mass spectrometry allow the simultaneous determination of tens, or even hundreds, of target analytes. In such cases, the traditional approach to quantitative method validation presents three major drawbacks: (i) it is extremely laborious, repetitive and rigid; (ii) it does not allow to introduce new target analytes without starting the validation from its very beginning and (iii) it is performed on spiked blank matrices, whose very nature is significantly modified by the addition of a large number of spiking substances, especially at high concentration. In the present study, several predictive chemometric models were developed from closed sets of analytes in order to estimate validation parameters on molecules of the same class, but not included in the original training set. Retention time, matrix effect, recovery, detection and quantification limits were predicted with partial least squares regression method. In particular, iterative stepwise

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Prediction of parameters
Ultra-high performance liquid chromatography–tandem mass spectrometry

elimination, iterative predictors weighting and genetic algorithms approaches were utilized and compared to achieve effective variables selection. These procedures were applied to data reported in our previously validated ultra-high performance liquid chromatography–tandem mass spectrometry multi-residue method for the determination of pharmaceutical and illicit drugs in oral fluid samples in accordance with national and international guidelines. Then, the partial least squares model was successfully tested on naloxone and lormetazepam, in order to introduce these new compounds in the oral fluid validated method, which adopts reverse-phase chromatography. Retention time, matrix effect, recovery, limit of detection and limit of quantification parameters for naloxone and lormetazepam were predicted by the model and then positively compared with their corresponding experimental values. The whole study represents a proof-of-concept of chemometrics potential to reduce the routine workload during multi-residue methods validation and suggests a rational alternative to ever-expanding procedures progressively drifting apart from real sample analysis.

1. Introduction

Validation procedures for the analytical methods are devoted to evaluate their performance and applicability in agreement with the declared objectives. Validation procedures alternatively refer to official methods (reported in national or international journals), normed methods (developed by International (ISO), European (CEN) or National (UNI) regulatory agencies), published methods (by agencies or approved associations), and internal methods (fully developed in the laboratory). In all these circumstances the validation process follows common criteria, accepted by the scientific community [1]. The validation of analytical methods represents nowadays one of the most important assignments within the laboratory activity, as all methods have to be tested, in order to verify their fulfillment of the expected objectives and performances. First, it is necessary to prove that each procedure is adequate and applicable to the analytical query under examination. Then, the operators' competence has to be tested by verifying suitable quality parameters. At last, sufficient data has to be collected to define control values and their confidence intervals, in order to make easy verification of the subsistence of quality parameters during daily routine work possible.

Among internal methods, a multitude of ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) protocols has been recently developed to simultaneously detect a wide variety of target analytes in biological samples, such as whole blood, oral fluid, urine and hair [2–10]. For such applications, UHPLC-MS/MS methods gradually replace gas chromatography–mass spectrometry (GC–MS), because of their extended applicability to polar analytes (without derivatization), higher sensitivity, and shorter analysis time, finally leading to increased productivity. Reliable validation of these methods requires the estimation of many experimental parameters, defined in European and International guidelines [11–13]. These include accuracy (consisting of trueness and precision), selectivity, specificity, linearity (linear dynamic range), limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility, robustness, recovery and matrix effect [13–15]. Moreover, the efficiency and reliability of newly built methods depend on the development and optimization of their operating conditions [13,16].

A large number of experiments and calculations has to be carried out during the validation process of UHPLC-MS/MS multi-residue methods. This systematic work is laborious and time consuming, and often hampers the routine laboratory work. Moreover, the introduction of new analytes into an existing and validated method is frequently required: for instance, in forensic and clinical toxicology, the analytical laboratory had recently to deal with the penetration of new or modified pharmaceutical and illicit drugs (i.e., synthetic cannabinoids, cathinones, amphetamines, opioids, benzodiazepines) into the legal and illegal markets [17,18]. The traditional approach to method validation does not allow the introduction of new target analytes into an existing protocol without starting the validation process from its very beginning, because of the potential interactions and reciprocal influences of the target analytes on the validation parameters. Obviously, this requirement represents a serious limitation to the frequent update of procedures and involves once again a considerable consumption of time and money. The recent definition of "open validation protocols" tries to overcome these limitations and encourages the proposal of innovative and intelligent approaches to the demanding need of reliable validation procedures [1,19].

Aim of the present study was to find out feasible and reliable chemometric procedures to evaluate and predict validation and chromatographic parameters (i.e., retention time (RT), matrix effect (ME), extraction recovery (ER), LOD, and LOQ), in order to overcome the prevalent drawbacks of the traditional procedure for quantitative method validation, particularly in multi-residue UHPLC-MS/MS methods. Furthermore, the applicability of this chemometric approach to investigate the introduction of new substances in already validated analytical methods was verified.

2. Materials and methods

2.1. UHPLC-MS/MS data

In previous studies conducted in our laboratory [4,6,20], multi-residue UHPLC-MS/MS methods were developed and fully-validated in accordance with ISO/IEC 17025:2005 requirements, to simultaneously detect several pharmaceutical and illicit drugs in various biological matrices (oral fluid, hair, and blood). In the present study, we reconsidered in particular our multi-residue UHPLC-MS/MS methods on oral fluid [4]. The target analytes were licit and illicit drugs (and metabolites) with psychotropic effects, most frequently prescribed or abused in the Italian territory. These drugs include, e.g., 4-hydroxyalprazolam, 6-monoacetylmorphine (6-MAM), 7-aminoclonazepam, 7-aminonitrazepam, alprazolam, amphetamine, amytriptyline, bromazepam, buprenorphine, carbamazepine, chlorpromazine, clonazepam, cocaine, codeine, delorazepam, desalkylflurazepam, diazepam, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), fentanyl, flunitrazepam, fluoxetine, flurazepam, ketamine, lorazepam, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), methadone, methamphetamine, midazolam, morphine, nitrazepam, norbuprenorphine, nordiazepam, norfentanyl, olanzapine, oxcarbamazepine, oxycodone, paroxetine, quetiapine, tetrahydrocannabinol, tramadol, triazolam, venlafaxine and zolpidem [4]. All experimental details, including the origin of the chemicals used, reagents, reference standards, biological specimen pre-treatments, sample preparations, instrumentations and method validation