Sudarshan Publications

Available online at https://s-epub.in/index.php/trp/index

Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2025.v17.i01.pp27-36

LC-MS/MS BIOANALYTICAL METHOD FOR QUANTIFICATION OF DAPAGLIFLOZIN IN HUMAN PLASMA

DUARTE PIMENTEL[1]*, ELEANOR POLGLASE[2], KONSTANTINOS MAVRIDIS[3]

Affiliation

[1]Department of Pharmaceutical Analysis, Douro Valley Institute of Pharmaceutical Sciences, Vila Real, Portugal
[2]Department of Quality Assurance, Exmoor Institute of Pharmaceutical Sciences, Taunton, United Kingdom
[3]Department of Pharmaceutical Analysis, Macedonia Plain Institute of Pharmacy, Serres, Greece

*Corresponding author: Duarte Pimentel, d.pimentel@dvips.pt
ORCID iD: Duarte Pimentel 0000-0001-0679-5017; Eleanor Polglase 0000-0001-0682-2740; Konstantinos Mavridis 0000-0001-4561-0396

Article info

Received date:26-05-2024
Accepted date:29-09-2024
Published date:31-01-2025
ISSN;0975-8216

Keywords:

Dapagliflozin, LC-MS/MS, Bioanalysis, Method validation, Human plasma

Abstract

Dapagliflozin, a sodium-glucose co-transporter 2 inhibitor now used well beyond diabetes, is routinely measured in human plasma by liquid chromatography with tandem mass spectrometry (LC-MS/MS) for pharmacokinetic, bioequivalence and interaction studies. This narrative review summarises what published work and regulatory guidance say about designing and validating such an assay: sample clean-up, chromatographic separation, ionisation and detection, internal standards, matrix effects, calibration, stability and incurred-sample checks. It follows the typical sequence of method development and discusses the weak points that recur. The review reports no new data. The main gap is that published methods differ in how fully they document matrix and metabolite effects.

1. INTRODUCTION

Dapagliflozin was the first sodium-glucose co-transporter 2 (SGLT2) inhibitor to reach the market in Europe and later in the United States, and its clinical reach has widened a great deal since. It lowers plasma glucose by blocking renal glucose reabsorption [1, 2], and clinical trials have since shown glycaemic benefit as well as benefit in heart failure and chronic kidney disease [3, 4, 5]. Each new indication brings more patients, more co-medication and more studies of

exposure in special populations. All of them rest on a plasma concentration measurement. The quality of the pharmacokinetic conclusion cannot exceed the quality of the assay beneath it, which is why method design deserves the attention that it gets in regulatory guidance [6, 7, 8].

Liquid chromatography coupled to tandem mass spectrometry has been the standard for small-molecule bioanalysis for roughly two decades. Jemal described its rise as a high-throughput technique, and Xu and colleagues later documented how faster chromatography, 96-well formats and automated liquid handling have shortened run times and increased capacity [9, 10]. For a drug such as dapagliflozin, with plasma concentrations that span a wide range between peak and trough, the combination of selectivity and sensitivity is hard to match with ultraviolet or fluorescence detection. The technique is not trouble free, though. Ion suppression, interference from metabolites and carry-over can all damage accuracy without producing any obvious warning in a quick inspection of chromatograms [11, 12].

A method for quantifying a drug in plasma is normally presented as a validation study. Here the topic is treated instead as a review of how such methods are built and judged, and the discussion reports no experimental work of its own. The material comes from regulatory guidelines from the US Food and Drug Administration, the European Medicines Agency and the International Council for Harmonisation, from pharmacokinetic studies of dapagliflozin, and from general bioanalytical literature on extraction, chromatography, ionisation and validation [6, 7, 8, 13]. Where a statement concerns dapagliflozin specifically it is limited to widely reported properties, with the aim of giving analysts a checklist of decisions that deserve explicit justification.

The sections that follow trace the workflow of a typical method. They begin with the properties of the analyte that drive early choices, move through sample preparation and separation, and then address detection, internal standards and matrix effects. A further section covers validation parameters and the practical evidence that regulators ask for, followed by a brief look at how validated assays have been applied in clinical pharmacology. Published findings and design choices are collected in table 1, and their distribution by method component is shown in fig. 1. The review ends with limitations and some suggestions for work that would make future assays easier to compare and to reproduce [14, 15].

Harmonisation has changed the working environment. Until recently, laboratories supporting global programmes had to satisfy the FDA and EMA documents separately, and the two differed in several details, such as the acceptance of incurred sample reanalysis criteria and the handling of ligand binding assays. Kaza and colleagues compared the 2018 FDA guidance with the EMA guideline and found most differences to be matters of emphasis, not of principle [15]. The ICH M10 guideline now aims to replace both with one text, which simplifies planning for multinational studies and reduces duplicated validation experiments [8, 16]. Smaller laboratories gain most, because they no longer need to maintain two sets of procedures for what is essentially one scientific question.

Table 1: Design choices in LC-MS/MS plasma assays and the published evidence that informs each

Method component	Design consideration reported	Ref.
Protein precipitation	Fast and suited to high throughput but leaves phospholipids; solvent and ratio alter ionisation	[17, 18]
Liquid-liquid extraction	Cleaner extracts for neutral drugs at the cost of extra handling	[19, 20]
Solid-phase extraction 96-well format	Best clean-up and recovery consistency; automation reduces labour	[20, 21]
Reversed-phase gradient	Volatile additives set adduct formation and peak shape; wash steps limit phospholipid build-up	[22, 23]
Electrospray ionisation	Droplet competition causes ion suppression that varies between individual samples	[12, 24, 25]
Labelled internal standard	Preferred, but may fail to track the analyte if separated or impure	[26, 27, 28]

Method component	Design consideration reported	Ref.
Three-set matrix experiment	Matrix factor and recovery from neat, post-spiked and pre-spiked samples	[29, 30]
Weighted calibration	Reciprocal concentration weighting improves accuracy at the low end	[31, 32]
Stability panel	Bench-top, freeze-thaw, long-term and processed stability must cover real study conditions	[6, 7, 13]
Incurred sample reanalysis	Repeat analysis of study samples tests behaviour spiked controls cannot show	[16, 33]

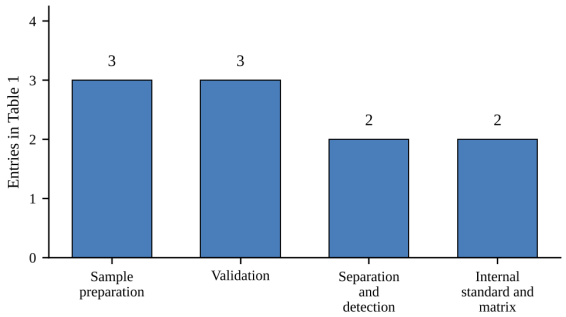


Fig. 1: Number of entries in table 1 by method component

2. ANALYTE PROPERTIES AND ANALYTICAL REQUIREMENTS

Dapagliflozin is a C-glucoside, which makes it more resistant to glycosidase hydrolysis than O-glucoside predecessors such as phlorizin. Its molecular weight is close to 409, it is moderately lipophilic and it carries no strongly ionisable group at physiological pH. Pharmacokinetic studies in healthy volunteers describe rapid oral absorption, high bioavailability, a terminal half-life of roughly half a day and extensive metabolism, chiefly by glucuronidation to an inactive 3-O-glucuronide [1, 34]. These properties set the analytical agenda: a neutral, moderately lipophilic parent drug is easy to extract from plasma into an organic solvent, but a more polar glucuronide metabolite will co-exist in samples and may interfere in some conditions.

The concentration range that the assay must cover follows from dosing and kinetics. Marketed doses are small, at the milligram level, and plasma concentrations after a single dose rise to a peak within a couple of hours and then decline over a day, so the lower limit of quantification must be low enough to capture the terminal phase [1, 2]. Studies with renal impairment, where exposure to the glucuronide rises and the pharmacodynamic response weakens, call for good selectivity against metabolites [35]. Interaction studies with enzyme inducers or inhibitors, such as the work with rifampicin and mefenamic acid, require an assay that behaves consistently across subjects who may have very different clearance values [36].

Food and fasting status can alter sampling plans. A study of a high-fat meal found a modest delay in the time to peak and a reduction in peak concentration, with little change in overall exposure, so a method must be able to define a sharp, early peak accurately in fasted subjects and a lower, later peak in fed ones [37]. For bioequivalence studies, regulators expect complete concentration-time profiles, and many protocols use sampling schedules that extend to several half-lives of the parent drug. This sets the dynamic range needed for calibration, typically spanning three orders of magnitude, and has implications for how dilution of over-range samples must be validated [2, 6, 7].

The analyte-related stability profile also matters at the planning stage. The glucuronide can, in principle, revert to the parent compound under certain conditions in the sample or in the ion source, which would artificially raise the measured parent concentration. This behaviour is well known for acyl glucuronides and, in a milder form, for other conjugates, and it justifies chromatographic separation of the metabolite from the parent rather than reliance on mass selectivity alone [13, 38]. Anticipating such issues from the structure of the drug saves time later, since the discovery of an in-source conversion problem late in validation can force a change of mobile phase or column and a repeat of earlier experiments [29, 30].

Clinical use also affects what is asked of the assay. Dapagliflozin is prescribed to patients who often take metformin, antihypertensives, statins, diuretics and insulin, and any of these might produce interfering peaks or ion

suppression in a real sample. Interaction studies in healthy subjects found no clinically relevant pharmacokinetic effects with several co-medications, and this is reassuring, yet absence of an effect on exposure is different from absence of analytical interference [36, 37]. Selectivity experiments conventionally use blank plasma from at least six individual sources, plus hyperlipidaemic and haemolysed samples, and a pragmatic team adds the common co-medications of the target population to that list [6, 7, 8].

3. SAMPLE PREPARATION AND CHROMATOGRAPHIC SEPARATION

Three extraction approaches account for most published plasma assays: protein precipitation, liquid-liquid extraction and solid-phase extraction. Reviews of sample preparation describe the compromises involved [19, 20]. Protein precipitation with acetonitrile or methanol is quick and cheap, and it suits high-throughput work, but it leaves most phospholipids in the extract and so invites matrix effects. Polson and colleagues compared precipitants and showed that the choice of solvent and plasma-to-solvent ratio altered both protein removal and ionisation effects [17]. For a lipophilic neutral drug, precipitation can be adequate if chromatography moves the analyte clear of the phospholipid elution zone, but the point needs to be demonstrated, not assumed [17, 18].

Liquid-liquid extraction into a water-immiscible solvent such as methyl tert-butyl ether or ethyl acetate gives cleaner extracts for neutral drugs. The partition removes most salts, proteins and polar phospholipid head groups, and the evaporated extract can be reconstituted in a small volume of mobile phase, concentrating the analyte. The trade-off is the extra handling, which makes the process harder to automate and adds opportunities for loss. Solid-phase extraction, usually on a polymeric reversed-phase sorbent, offers the most thorough clean-up and the best recovery consistency, at higher cost per sample [19, 20]. Henion and colleagues described how the 96-well format brought solid-phase extraction into routine bioanalysis, and automation now removes much of the labour penalty [21].

Chromatography has two jobs: to separate the analyte from isobaric interferences and to move it away from the zone where suppressing matrix components elute. Reversed-phase C18 columns with sub-3 µm particles are used in nearly all reported methods, with water and acetonitrile or methanol gradients and a volatile additive such as ammonium acetate or formic acid. The additive does more than control retention. It supplies the ions that form adducts in the source, so the choice affects sensitivity as well as peak shape. Kostiainen and Kauppila reviewed how eluent composition influences ionisation efficiency, and their conclusions apply directly to the choice of mobile phase for a neutral glycoside [22, 23].

Run time is the main lever for throughput. Analytical runs of two to four minutes per sample are common in published methods for small molecules, achieved with short columns and steep gradients, and ultra-high-pressure systems can reduce them further [9, 10]. Shortening the run increases the risk that phospholipids from one injection elute during the next, an effect which shows up as drifting responses across a batch. Many laboratories therefore include a strong organic wash after each gradient, or divert the early and late eluent to waste. A well-designed method treats the column as part of the clean-up, and monitors a phospholipid transition during development to confirm that the analyte peak is free of overlap [18, 39].

Carry-over is a particular concern with sensitive assays that cover a wide range. If a high-concentration sample leaves residue in the autosampler needle or the column, the following blank can show a peak. Regulatory guidance sets an acceptance limit relative to the lower limit of quantification, and requires that the effect be assessed and minimised during validation [6, 7]. Practical remedies include strong needle-wash solvents, injection of blanks after high samples and the arrangement of the run order so that samples from the same subject are bracketed sensibly. The issue is easy to overlook in developing a method, because early work uses calibrators and quality controls which rarely approach the concentration of a real peak sample [6, 8, 13].

Sample volume is another design variable. Many assays use 100 to 500 µL of plasma, and smaller volumes help in studies with frequent sampling or in paediatric work. Lower volumes demand a more sensitive detector and often a more concentrated extract, which can increase matrix effects. Microsampling methods, including dried blood spots, have been explored for several drugs, but they bring their own validation issues, such as haematocrit effects, and are not interchangeable with plasma without a formal bridging study [19, 20]. Deciding whether the added convenience justifies the extra validation is a matter for the study team, and the answer depends on how often samples are taken and by whom.

4. MASS SPECTROMETRIC DETECTION AND INTERNAL STANDARDS

Electrospray ionisation is the usual interface, and its mechanism explains many of the practical problems analysts meet. Kebarle and Tang described how charged droplets shrink and release gas-phase ions, and how analytes compete for limited surface charge on the droplet [25]. In a crowded droplet, a co-eluting compound that is more surface-active will suppress ionisation of the analyte. Buhrman and colleagues documented this ion suppression effect in an early quantitative study of a drug in human plasma, long before it was routinely tested for [24]. Annesley summarised the phenomenon for clinical laboratories and stressed that suppression varies between individual samples, so a single pooled matrix check is not enough [12].

Dapagliflozin lacks a basic nitrogen, so protonation is not favoured. Reported methods typically exploit adduct formation, with the ammonium adduct in positive mode being a frequent choice, while negative mode with a formate or acetate adduct has also been used. The selection depends on the mobile phase additive, on the stability of the precursor ion and on the background noise at the chosen transition [22, 23]. Product ions are generated by collision-induced dissociation, usually through loss of the sugar moiety or fragments of it, and selected reaction monitoring then gives selectivity. Analysts are advised to monitor a second transition during development, since a ratio that stays constant between standards and real samples provides additional evidence that no interference is present [13, 38].

An internal standard corrects for variation in extraction recovery, injection volume and ionisation. The preferred choice is a stable isotope-labelled version of the analyte, because it co-elutes and so experiences the same matrix environment. Stokvis and colleagues reviewed the evidence and concluded that a labelled standard is the best option where it can be obtained, though structural analogues can work if they are chosen with care [26]. Isotope-labelled analogues of dapagliflozin can be made, and a carbon-13 version would avoid the slight retention shift that deuterium can cause. Choosing a standard that elutes at a different time from the analyte, by contrast, removes most of the value of the correction [26, 28].

Even labelled standards do not guarantee correction. Wang and colleagues described cases in which a stable isotope-labelled standard failed to track the analyte, because of a small chromatographic separation between them, differences in purity or interference from the labelled material in the analyte channel [27]. Hewavitharana argued that matrix matching of calibrators is less necessary when a labelled standard is used, yet he did not claim it to be unnecessary in every case [28]. Checks that detect these problems include monitoring the internal standard response across the run, looking for isotopic cross-talk in blank samples spiked only with internal standard, and testing the analyte at the lower limit with the standard at its working concentration [26, 27].

Quantitation of matrix effects is explicit in modern guidance. Matuszewski and colleagues proposed a three-set experiment: neat solutions, blank extracts spiked after extraction and samples spiked before extraction. From these the matrix factor, recovery and process efficiency are calculated, and the internal standard-normalised matrix factor should show a coefficient of variation within the limit set by the guideline across at least six lots of matrix [6, 7, 29]. A later paper from

the same group suggested comparing calibration line slopes in different matrices as an alternative measure of relative matrix effect [30]. Chambers and colleagues offered a systematic strategy for reducing matrix effects, in which extraction, chromatography and ionisation are each considered in turn [18].

5. VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

Validation practice has a long lineage. The 1990 Washington conference and the 1992 report by Shah and colleagues set out the first widely accepted parameters for bioavailability and bioequivalence studies [40]. A decade later, a second report revisited them in the light of experience and introduced the concept of reproducing results in incurred samples [14]. The Crystal City meetings refined the picture further, and the 2013 revised draft of the FDA guidance reflected that discussion [16]. The core parameters have changed little: selectivity, calibration, accuracy, precision, recovery, matrix effect, carry-over, dilution integrity and stability. Current documents differ mostly in how matrix effects and incurred sample reanalysis are handled [6, 7, 8, 15].

Calibration is the first quantitative parameter. A blank, a zero sample and at least six non-zero calibrators across the range are expected, and a regression model is chosen to match the response. In LC-MS/MS the variance of the response grows with concentration, so an unweighted least-squares line gives undue influence to the high standards and poor accuracy at the low end. Almeida and colleagues reviewed weighting schemes for calibration lines and showed the benefit of weighting by the reciprocal of concentration or its square [31]. Gu and colleagues later showed how choosing the wrong weighting factor degrades curve stability and data quality, and recommended a statistical rather than visual selection [32].

Accuracy and precision are assessed with quality control samples at the lower limit of quantification, low, medium and high levels, analysed in replicate over several runs. Guidance asks for within-run and between-run results to fall within 15% of nominal, and within 20% at the lower limit, with the precision criteria set to match [6, 7, 8]. These limits are well known, and so is the trap behind them: passing the criteria in a validation does not guarantee performance in a routine batch with dozens of subject samples. Bansal and DeStefano pointed out that the quality control samples should mimic study samples as far as possible, using the same matrix and storage, so that the validation reflects what will happen in practice [38].

Stability must be demonstrated under every condition that study samples will meet. That means bench-top stability at room temperature, freeze-thaw cycles, long-term frozen storage, processed-sample stability in the autosampler and stock solution stability. The storage period validated must cover the time from collection to analysis in the real study, or the results cannot be defended. For dapagliflozin and its glucuronide, the practical risk concerns possible conversion of the conjugate, so the stability experiments should be run in the presence of the metabolite and not only with the parent in a clean matrix [6, 7, 13]. Whole-blood stability is sometimes added when samples are not centrifuged immediately after collection [8, 13].

Incurred sample reanalysis tests whether the method works on real study samples, in which protein binding, metabolites and co-medications differ from those in spiked quality controls. The Crystal City recommendations, discussed in a follow-up workshop report, call for repeat analysis of a proportion of samples around the peak and in the elimination phase, with at least two thirds of the repeats within 20% of the original [33]. Failures typically point to problems that the spiked samples cannot reveal, such as metabolite back-conversion, uneven sample homogeneity or protein-binding differences. The requirement has been retained in the newer harmonised text, so it is now a standard part of the validation and study routine [8, 16, 33].

Documentation is a validation topic in its own right. Reports should list the lots of matrix, the dates and instrument identity, and give the complete set of raw chromatograms for failed

as well as passed runs, since regulators inspect the reasons why a run was rejected. Reintegration of peaks must follow a written procedure and be justified, and all versions of an integration should remain in the audit trail. Laboratories under good laboratory practice or good clinical practice oversight are inspected on these aspects at least as closely as on the numerical results [6, 13, 16].

6. APPLICATION IN PHARMACOKINETIC AND CLINICAL STUDIES

Validated assays have underpinned the clinical pharmacology of dapagliflozin from the first human studies. Dose-ranging work in healthy subjects related the amount of glucose excreted in urine to dose, and the pharmacokinetic-pharmacodynamic relationship that emerged guided the choice of marketed doses [2]. Studies in renal impairment showed that exposure to the parent and glucuronide increased with decreasing kidney function, while the glucose-lowering effect declined, results that depended on precise measurement over a range of concentrations [35]. A comprehensive review of the pharmacokinetics and pharmacodynamics of the drug brought these findings together, and it is a useful companion to any analytical paper on the compound [1].

Bioequivalence work for generic products creates the heaviest analytical workload. A typical study analyses thousands of samples from a few dozen subjects, in two or more periods, and the assay must remain stable over the weeks or months that this takes. The expectation is that the lower limit of quantification allows the profile to be followed for at least three half-lives, so that the area under the curve can be extrapolated with confidence [6, 7]. Because the primary comparison depends on ratios of exposure measures with a narrow confidence interval, small systematic biases from a drifting method can matter more than random imprecision, which is another reason to monitor internal standard response across the batch [9, 27]. Reviewers of such studies commonly ask for the batch layout, the reasons for any rejected run and the internal standard trend as part of the bioanalytical report, and a laboratory that keeps them from the start finds the request easy to meet [16, 33].

Therapeutic drug monitoring is not part of routine dapagliflozin care, because its wide therapeutic index and the weak link between plasma concentration and glycaemic effect reduce the need. Nonetheless, there are special situations in which concentration data help: investigation of adherence, studies in children, suspected interactions and research in organ failure or critical illness. Assays for such work may need to accommodate unusual matrices, such as haemolysed or lipaemic plasma, and small sample volumes [20, 35, 36]. Wider use of the drug in heart failure and kidney disease populations, where older age and multiple medicines are the norm, makes a robust and well-characterised assay more valuable than before [4, 5].

7. LIMITATIONS AND FUTURE DIRECTIONS

This synthesis has limits that readers should keep in mind. It rests on guidance documents, general bioanalytical literature and clinical pharmacology papers, and it does not compare the performance of individual published dapagliflozin assays head to head. Published method papers vary in how fully they report matrix factors across lots, behaviour of the glucuronide and carry-over, so a conclusion that one reported assay is better than another cannot be drawn from the papers alone [13, 15, 29]. Table 1 therefore lists design choices and the evidence that supports each, instead of rating methods. Readers needing a specific validated procedure should consult the primary papers and the relevant guidance in full [6, 7, 8].

Several practical gaps remain. Reporting of incurred sample reanalysis and of metabolite interference is inconsistent, and the proportion of published assays that test more than one matrix lot for matrix factor is not well documented. Cross-laboratory reproducibility is rarely examined, although it is central when data from different sites are pooled [15, 33]. A second concern is the assumption that a labelled internal standard resolves all matrix issues, which Wang and colleagues showed to be too optimistic in

some cases [27]. Closer attention to these points would raise confidence in pharmacokinetic conclusions drawn from the assay, especially in special populations where matrix composition departs from that of healthy volunteers [12, 18]. Patients with kidney or liver disease, for instance, may carry different levels of lipids, bilirubin and urea in plasma, and these can shift ion suppression in ways that healthy-volunteer validation never exposes.

Future developments are likely to centre on higher-resolution instruments, on smaller sample volumes and on greater automation. High-resolution mass spectrometers offer selectivity advantages and allow retrospective data mining for metabolites, but their quantitative performance across wide ranges must be proved against the tandem quadrupole standard [10, 23]. Multiplexed assays that measure several SGLT2 inhibitors or co-prescribed drugs in one run could help in interaction studies and in clinical settings. Harmonisation under ICH M10 should reduce duplication of effort, provided that laboratories apply it consistently, and shared reporting templates would make published methods easier to compare [8, 15, 16].

8. CONCLUSION

A dependable LC-MS/MS assay for dapagliflozin in human plasma is the product of many small decisions, each of which has a published rationale. A neutral glycoside with a major glucuronide metabolite calls for clean extraction, chromatography that separates metabolite from parent, an adduct-based ionisation scheme chosen deliberately, and a co-eluting labelled internal standard whose behaviour is checked and not assumed [26, 27, 34]. The regulatory texts agree on the validation parameters and are converging on how to apply them, with matrix factor from several lots, weighted calibration, a full stability panel and incurred sample reanalysis as the main safeguards [6, 8, 29, 32, 33].

For analysts, the evidence suggests spending effort early on matrix effects and metabolite interference, since problems found late are expensive to correct [12, 18]. For readers of published assays, the message is to look for evidence of these checks before trusting reported pharmacokinetic results, particularly in special populations and bioequivalence work [15, 35]. Reporting that is more complete, shared validation templates and clearer data on cross-laboratory performance would help the field. As use of the drug expands in heart failure and kidney disease, the quality of the underlying concentration data will matter more, not less [4, 5].

9. ABBREVIATIONS

EMA-European Medicines Agency, FDA-US Food and Drug Administration, ICH-International Council for Harmonisation, LC-MS/MS-Liquid chromatography with tandem mass spectrometry, SGLT2-Sodium-glucose co-transporter 2, QC-Quality control, LLOQ-Lower limit of quantification, ISR-Incurred sample reanalysis, SPE-Solid-phase extraction, LLE-Liquid-liquid extraction

10. FUNDING

Nil

11. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

1. Kasichayanula S, Liu X, Lacreta F, Griffen SC, Boulton DW. Clinical pharmacokinetics and pharmacodynamics of dapagliflozin, a selective inhibitor of sodium-glucose co-transporter type 2. Clin Pharmacokinet 2014;53:17–27.
2. Komoroski B, Vachharajani N, Boulton D, Kornhauser D, Geraldes M, Li L, *et al.* Dapagliflozin, a novel SGLT2 inhibitor, induces dose-dependent glucosuria in healthy subjects. Clin Pharmacol Ther 2009;85:520–526.
3. Bailey CJ, Gross JL, Pieters A, Bastien A, List JF. Effect of dapagliflozin in patients with type 2 diabetes who have inadequate glycaemic control with metformin: a randomised, double-blind, placebo-controlled trial. Lancet 2010;375:2223–33.
4. Solomon SD, McMurray JJV, Claggett B, de Boer RA, DeMets D, Hernandez AF, *et al.* Dapagliflozin in heart failure with mildly reduced or preserved ejection fraction. N Engl J Med 2022;387:1089–98.

5. Wheeler DC, Stefánsson BV, Jongs N, Chertow GM, Greene T, Hou FF, *et al.* Effects of dapagliflozin on major adverse kidney and cardiovascular events in patients with diabetic and non-diabetic chronic kidney disease: a prespecified analysis from the DAPA-CKD trial. Lancet Diabetes Endocrinol 2021;9:22–31.

6. FDA. Bioanalytical method validation: guidance for industry. Rockville: US Department of Health and Human Services; 2018.

7. EMA. Guideline on bioanalytical method validation. London: European Medicines Agency; 2011.

8. ICH. M10 Bioanalytical method validation and study sample analysis. Geneva: ICH; 2022.

9. Jemal M. High-throughput quantitative bioanalysis by LC/MS/MS. Biomed Chromatogr 2000;14:422–429.

10. Xu RN, Fan L, Rieser MJ, El-Shourbagy TA. Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS. J Pharm Biomed Anal 2007;44:342–355.

11. Bonfiglio R, King RC, Olah TV, Merkle K. The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds. Rapid Commun Mass Spectrom 1999;13:1175–1185.

12. Annesley TM. Ion suppression in mass spectrometry. Clin Chem 2003;49:1041–1044.

13. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, *et al.* Workshop/conference report: quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. AAPS J 2007;9:E30–E42.

14. Shah VP, Midha KK, Findlay JWA, Hill HM, Hulse JD, McGilveray IJ, *et al.* Bioanalytical method validation, a revisit with a decade of progress. Pharm Res 2000;17:1551–1557.

15. Kaza M, Karaźniewicz-Łada M, Kosicka K, Siemiątkowska A, Rudzki PJ. Bioanalytical method validation: new FDA guidance vs. EMA guideline. Better or worse? J Pharm Biomed Anal 2019;165:381–385.

16. Booth B, Arnold ME, DeSilva B, Amaravadi L, Dudal S, Fluhler E, *et al.* Workshop report: Crystal City V quantitative bioanalytical method validation and implementation: the 2013 revised FDA guidance. AAPS J 2015;17:277–288.

17. Polson C, Sarkar P, Incledon B, Raguvaran V, Grant R. Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography-tandem mass spectrometry. J Chromatogr B 2003;785:263–275.

18. Chambers E, Wagrowski-Diehl DM, Lu Z, Mazzeo JR. Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses. J Chromatogr B 2007;852:22–34.

19. Nováková L, Vlcková H. A review of current trends and advances in modern bio-analytical methods: chromatography and sample preparation. Anal Chim Acta 2009;656:8–35.

20. Kole PL, Venkatesh G, Kotecha J, Sheshala R. Recent advances in sample preparation techniques for effective bioanalytical methods. Biomed Chromatogr 2011;25:199–217.

21. Henion J, Brewer E, Rule G. Sample preparation for LC/MS/MS: analyzing biological and environmental samples. Anal Chem 1998;70:650A–656A.

22. Kostiainen R, Kauppila TJ. Effect of eluent on the ionization process in liquid chromatography-mass spectrometry. J Chromatogr A 2009;1216:685–699.

23. Hopfgartner G, Bourgogne E. Quantitative high-throughput analysis of drugs in biological matrices by mass spectrometry. Mass Spectrom Rev 2003;22:195–214.

24. Buhrman DL, Price PI, Rudewicz PJ. Quantitation of SR 27417 in human plasma using electrospray liquid chromatography-tandem mass spectrometry: a study of ion suppression. J Am Soc Mass Spectrom 1996;7:1099–1105.

25. Kebarle P, Tang L. From ions in solution to ions in the gas phase: the mechanism of electrospray mass spectrometry. Anal Chem 1993;65:972A–986A.

26. Stokvis E, Rosing H, Beijnen JH. Stable isotopically labeled internal standards in quantitative bioanalysis using liquid chromatography/mass spectrometry: necessity or not? Rapid Commun Mass Spectrom 2005;19:401–407.

27. Wang S, Cyronak M, Yang E. Does a stable isotopically labeled internal standard always correct analyte response? J Pharm Biomed Anal 2007;43:701–707.

28. Hewavitharana AK. Matrix matching in liquid chromatography-mass spectrometry with

stable isotope labelled internal standards: is it necessary? J Chromatogr A 2011;1218:359–361.

29. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 2003;75:3019–3030.
30. Matuszewski BK. Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis. J Chromatogr B 2006;830:293–300.
31. Almeida AM, Castel-Branco MM, Falcão AC. Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods. J Chromatogr B 2002;774:215–222.
32. Gu H, Liu G, Wang J, Aubry AF, Arnold ME. Selecting the correct weighting factors for linear and quadratic calibration curves with least-squares regression algorithm in bioanalytical LC-MS/MS assays and impacts of using incorrect weighting factors on curve stability, data quality, and assay performance. Anal Chem 2014;86:8959–8966.
33. Fast DM, Kelley M, Viswanathan CT, O'Shaughnessy J, King SP, Chaudhary A, *et al.* Workshop report and follow-up: AAPS workshop on current topics in GLP bioanalysis: assay reproducibility for incurred samples, implications of Crystal City recommendations. AAPS J 2009;11:238–241.
34. Obermeier M, Yao M, Khanna A, Koplowitz B, Zhu M, Li W, *et al.* In vitro characterization and pharmacokinetics of dapagliflozin (BMS-512148), a potent sodium-glucose cotransporter type II inhibitor, in animals and humans. Drug Metab Dispos 2010;38:405–414.
35. Kasichayanula S, Liu X, Pe Benito M, Yao M, Pfister M, LaCreta FP, *et al.* The influence of kidney function on dapagliflozin exposure, metabolism and pharmacodynamics in healthy subjects and in patients with type 2 diabetes mellitus. Br J Clin Pharmacol 2013;76:432–444.
36. Kasichayanula S, Liu X, Griffen SC, Lacreta FP, Boulton DW. Effects of rifampin and mefenamic acid on the pharmacokinetics and pharmacodynamics of dapagliflozin. Diabetes Obes Metab 2013;15:280–283.
37. Kasichayanula S, Liu X, Zhang W, Pfister M, Reele SB, Aubry AF, *et al.* Effect of a high-fat meal on the pharmacokinetics of dapagliflozin, a selective SGLT2 inhibitor, in healthy subjects. Diabetes Obes Metab 2011;13:770–773.
38. Bansal S, DeStefano A. Key elements of bioanalytical method validation for small molecules. AAPS J 2007;9:E109–E114.
39. Taylor PJ. Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. Clin Biochem 2005;38:328–334.
40. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, *et al.* Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. J Pharm Sci 1992;81:309–312.