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ANALYTICAL QUALITY BY DESIGN APPROACH TO A GREEN CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF A FIXED-DOSE COMBINATION: METHOD GREENNESS ASSESSMENT USING AGREE AND BLUE APPLICABILITY GRADE INDEX

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ABSTRACT

Background. Empagliflozin and linagliptin are co-formulated as a fixed-dose combination, and many liquid chromatographic assays have been published for it. Few were developed under the enhanced approach of ICH Q14, and none has been judged against both greenness and practicality metrics. **Objective.** To apply an Analytical Quality by Design (AQbD) workflow in silico to the simultaneous chromatographic assay of empagliflozin and linagliptin, benchmark published methods with the Analytical GREEnness metric (AGREE) and the Blue Applicability Grade Index (BAGI), and define a greenness design space for a replacement method. **Methods.** Ten peer-reviewed methods were coded under uniform rules and scored with a re-implementation of AGREE that reproduced the published case studies, and with BAGI. Retention factors were estimated from reported column geometry. A failure mode and effects analysis ranked method parameters, AGREE was mapped over flow rate and run time under a retention constraint, and robustness to coding assumptions was tested by Monte Carlo simulation (5000 draws). **Results.** AGREE scores of published methods ranged from 0.39 to 0.50 (median 0.45) and BAGI from 80 to 82.5, with no rank correlation between the two (Spearman rho 0.18, $p = 0.621$). Only one of nine evaluable methods gave an estimated retention factor of at least 2 for the first-eluting drug; six gave values below 0.5. Mobile-phase pH (risk priority number 60) and organic modifier (40) were the dominant risks. An ethanol-phosphate design on a 100 x 2.1 mm column reached AGREE 0.59 and BAGI 82.5 with 1.2 mL of mobile phase per run; both ethanol designs exceeded every published method in 92.9% of simulations. **Conclusion.** Greenness of this assay is governed by column volume, sample-preparation solvent and modifier toxicity, not by flow rate. The proposed operating region is a testable hypothesis that now requires laboratory verification and ICH Q2(R2) validation.

Keywords: analytical quality by design; green analytical chemistry; AGREE; blue applicability grade index; empagliflozin; linagliptin; method operable design region; RP-HPLC.

1. Introduction

Fixed-dose combinations place an awkward demand on the quality-control laboratory. Two active substances with different chemistry must be separated and quantified in one run, the ratio between them is often large, and the method must stay reliable across thousands of batches over a product lifetime. The combination of empagliflozin, a sodium-glucose co-transporter 2 inhibitor, with linagliptin, a dipeptidyl peptidase-4 inhibitor, shows this well. The product was approved in the United States in 2015 as 10/5 mg and 25/5 mg tablets [1], and in the European Union in 2016 [2], so linagliptin is always present at 5 mg while empagliflozin is present at two to five times that amount. Both drug substances are classed as Biopharmaceutics Classification System class III [2]. Their computed lipophilicities are almost identical (XLogP3 of 2 for empagliflozin and 1.9 for linagliptin) [3,4], yet their acid-base behaviour differs sharply. Linagliptin carries a basic centre with a reported pKa of 8.6 [4], while empagliflozin, a C-glucoside, has no ionisable group in the working pH range of reversed-phase columns [3]. In a reversed-phase system their separation therefore depends largely on the ionisation state of linagliptin, which makes mobile-phase pH a dominant variable.

Regulatory expectations for method development have moved in the same period. ICH Q14 set out an enhanced approach that begins with an analytical target profile (ATP), links method parameters to performance through risk assessment and experimentation, and can define a method operable design region within which changes can be managed with less regulatory burden [5]. It is paired with the revised validation guideline ICH Q2(R2) [6], with the risk principles of ICH Q9(R1) [7] and with the design-space concepts first stated in ICH Q8(R2) [8]. Chromatographers call this workflow Analytical Quality by Design (AQbD). Reviews have described how it moves method robustness from a test performed at the end of development to a property designed in from the start [9-11]. The design space itself is best treated as a region of guaranteed probability rather than an overlap of mean response surfaces [12,13].

A second development has been the arrival of formal greenness metrics. The twelve principles of green analytical chemistry, summarised in the SIGNIFICANCE mnemonic [14], have been turned into several scoring tools: the Analytical Eco-Scale [15], the Green Analytical Procedure Index [16], and the Analytical GREEnness metric (AGREE) [17]. AGREE converts each of the twelve principles to a score between 0 and 1 and gives an overall score with a clock-like pictogram. It is continuous, it can be weighted, and it is transparent about which principles a method fails. White Analytical Chemistry argues that greenness alone is not enough, since a method must also be analytically sound and practical to use [18]. The Blue Applicability Grade Index (BAGI) was proposed to capture the practical dimension through ten attributes scored from 2.5 to 10 [19]. Recent pharmaceutical work increasingly reports AGREE and BAGI side by side [20-23].

Liquid chromatography is a large source of solvent waste in pharmaceutical analysis, and acetonitrile and methanol dominate reversed-phase mobile phases [24-26]. Ethanol is a frequently proposed substitute. It is ranked favourably in solvent selection guides [27,28] and can be produced from renewable feedstock. Its eluotropic strength is higher than that of methanol, but its UV cut-off (210 nm) and the viscosity of ethanol-water mixtures limit detection wavelength and column back-pressure [29].

For empagliflozin and linagliptin, the published chromatographic literature is extensive but uneven. Methods range from conventional C18 assays with acidified acetonitrile [30,31] to ultra-high-performance separations [32,33] and a recent central composite design optimisation [34]. AQbD designs have been reported for the ternary combination with metformin [35,36], and one ternary study reported several greenness indices [37]. No study appears to have examined the two-drug assay through an ATP-driven AQbD lens that treats greenness and practicality as critical attributes and applies AGREE and BAGI to the published methods under one set of rules.

The present work addresses that gap without laboratory experiments or biological material. Its aims were (i) to define an ATP and critical quality attributes for the simultaneous assay, (ii) to benchmark published methods with a validated re-implementation of AGREE and with BAGI, (iii) to test whether those methods meet a basic retention criterion, (iv) to rank method parameters by risk, and (v) to map a greenness design space and propose operating points for experimental verification.

2. Materials and methods

2.1. Study design and analytical target profile

The study is a computational, secondary analysis of published analytical procedures. No human or animal material or data were used. The ATP was defined as follows: the procedure must quantify empagliflozin and linagliptin simultaneously in the film-coated tablet at both marketed strengths, with accuracy of 98.0-102.0% of the true content and repeatability below 2.0% relative standard deviation, and must be stability-indicating. Critical quality attributes (CQAs) were taken from the FDA reviewer guidance on chromatographic methods [38]: retention factor of the first-eluting drug (k1) of at least 2, resolution of at least 2, tailing factor of 2 or less, and plate count above 2000. Run time, AGREE and BAGI were added as greenness and practicality attributes.

2.2. Retrieval and extraction of published methods

Peer-reviewed reports of chromatographic methods for the simultaneous determination of empagliflozin and linagliptin in bulk or tablets were retrieved from publisher sites, CrossRef and bibliographic indexes up to September 2026, with search strings combining the drug names with HPLC, UPLC, stability-indicating, quality by design and green. Twenty-three eligible records were identified. Ten were retained: nine two-drug liquid chromatographic methods [30,31,33,34,39-43] and one three-drug ultra-high-

performance method validated on the empagliflozin-linagliptin product [32]. Records were excluded when the chromatographic conditions could not be accessed, when flow rate was not reported, when only planar chromatography was described, when the matrix was a biological fluid, or when peer review could not be confirmed. Column dimensions, mobile phase, flow rate, run time, retention times, detection and sample preparation were extracted as reported (Table 1). Methods were labelled M1 to M10 in chronological order.

Table 1. Chromatographic conditions of the ten published methods, as reported in the source articles.

ID	Ref.	Technique	Column (mm)	Mobile phase (v/v)	Flow (mL/min)	tR EMPA / LINA (min)
M1	[32]	UHPLC-DAD	C18, 100 x 2.1	KH2PO4 pH 4 : MeOH 50:50	0.4	4.7 / 2.3
M2	[30]	HPLC-UV	C18, 250 x 4.6	0.1% HClO4 : ACN 60:40	1.0	2.05 / 4.10
M3	[31]	HPLC-UV	C18, 250 x 4.6	0.1% H3PO4 : ACN 60:40	1.0	2.139 / 2.759
M4	[39]	HPLC-FLD	C18, 150 x 4.6	TEA pH 4.5 : ACN, gradient	1.7	4.19 / 2.17
M5	[33]	UHPLC-UV	C18, NR	phosphate pH 4 : ACN 65:35	1.5	1.417 / 2.453
M6	[40]	HPLC-UV	C18, 250 x 4.6	MeOH : 0.1% H3PO4 45:55	1.0	2.692 / 5.012
M7	[41]	HPLC-PDA	C18, 100 x 2.1	MeOH : water 60:40	0.5	1.320 / 2.343
M8	[42]	HPLC-UV	C18, 250 x 4.6	phosphate pH 5 : MeOH 20:80	1.5	1.86 / 2.61
M9	[34]	HPLC-PDA	C18, 250 x 4.6	MeOH : ACN 90:10	1.0	2.900 / 4.174
M10	[43]	HPLC-UV	C18, 250 x 4.6	MeOH : water 80:20	1.2	2.67 / 4.34

ACN, acetonitrile; MeOH, methanol; TEA, triethylamine; NR, not reported. M1 also resolves metformin; M10 also resolves a linagliptin impurity eluting at 9.17 min.

2.3. AGREE scoring

The twelve AGREE transformations were re-implemented in Python from the original report [17]: the tabulated scores for principles 1, 3, 4, 5, 10 and 12, and equations 1, 3, 4 and 5 for sample amount, waste, analytes per hour and toxic reagent amount. The overall score was the weighted mean of the criterion scores with equal default weights. As a check, the three worked case studies in the original report were recomputed from the inputs given there.

Because the source articles report sample preparation inconsistently, uniform coding rules were applied to all methods so that differences in score would reflect the chromatography. Every method was coded as external pretreatment with a reduced number of steps (principle 1), off-line measurement (principle 3), six analytical steps (principle 4), manual and non-miniaturised preparation (principle 5), no derivatisation (principle 6), 0.2 g of tablet powder (principle 2) and 25 mL of preparation solvent per sample. Run time was the reported value or, if none was given, 1.2 times the retention time of the last peak. Waste per sample (principle 7) was flow rate multiplied by run time, plus preparation solvent and sample. Analytes per hour (principle

8) were two multiplied by 60 divided by run time. Energy (principle 9) used the categorical AGREE classes: 0.5 for liquid chromatography and 1.0 for ultra-high-performance systems [17]. Alcohols were treated as potentially bio-based: a score of 1 was given when methanol or ethanol with water were the only reagents, 0.5 when other reagents were also used, and 0 otherwise (principle 10). Methanol, acetonitrile and strong acids were treated as toxic, and their volume per sample, from both the mobile phase and the diluent, entered equation 5 (principle 11). Hazards counted for principle 12 were flammability of organic solvents, corrosivity of phosphoric, perchloric acid or triethylamine, and oxidising properties of perchloric acid.

2.4. BAGI scoring

BAGI was scored from the ten attributes and four levels defined by Manousi et al. [19] and summed to a total between 25 and 100. All methods were coded as quantitative (7.5), determining two compounds of different chemical classes (7.5), on instrumentation available in most laboratories (7.5), with 2 to 12 samples prepared at once (5.0), simple low-cost preparation (7.5), common reagents (10), no preconcentration (10), semi-automation through an autosampler (7.5) and at most 10 g of sample (10). Throughput (attribute 6) was 60 divided by run time, since batch preparation runs in parallel with the chromatography.

2.5. Retention-factor screening

Column dead time was estimated as t0 = eps x Vc / F, where Vc is the geometric column volume from the reported length and internal diameter, F the flow rate and eps the total porosity, taken as 0.65 with 0.55 and 0.70 as bounds [44]. Retention factors were k = (tR − t0)/t0 and selectivity alpha = k(last)/k(first). M5 was excluded because its column dimensions were not reported. To see what adequate retention would cost in greenness, each method was rescored with its run time extended to at least 1.2 x t0 x (1 + 3), the time needed to elute a last peak with k = 3.

2.6. Risk assessment

Potential sources of variability were grouped in an Ishikawa structure (instrument, column, mobile phase, sample preparation, detection) and ranked by failure mode and effects analysis in line with ICH Q9(R1) [7]. Severity, occurrence and detectability were scored on 1-5 ordinal scales by the authors, using the retention screening and the physicochemical properties above. The risk priority number (RPN) was their product, and RPN of 36 or more was classed as high risk. These scores are expert judgements, not measurements.

2.7. Greenness design space and design targets

AGREE was computed over a grid of flow rate (0.2-1.8 mL/min, step 0.05) and run time (1-15 min, step 0.25) for three column formats (250 x 4.6 mm and 150 x 4.6 mm on conventional liquid chromatography, and 100 x 2.1 mm on ultra-high-performance equipment) and three mobile-phase chemistries (acetonitrile with acid, methanol with phosphate buffer, ethanol with phosphate buffer). A grid point was kept only

when run time was at least 1.2 x t0 x (1 + k_last) with k_last = 3, which corresponds to k1 = 2 and alpha = 1.5. The organic fraction was fixed at 0.40 and the preparation solvent at 10 mL. Two design targets were then placed at the lowest feasible run time, rounded up to the nearest 0.5 min: G1 on the 150 x 4.6 mm column at 1.0 mL/min and G2 on the 100 x 2.1 mm column at 0.4 mL/min, both with ethanol and unadjusted potassium dihydrogen phosphate.

2.8. Monte Carlo robustness analysis

The effect of the coding rules was tested with 5000 Monte Carlo draws (NumPy random generator, seed 20260923). In each draw, sample mass was drawn from U(0.05, 0.5) g, preparation solvent from U(10, 100) mL, the number of steps from 5, 6 or 7, and the run-time multiplier for methods without a reported run time from U(1.1, 1.5). Criterion weights came from a symmetric Dirichlet distribution with concentration 20 per criterion, rescaled to a mean of 1. For G1 and G2, preparation solvent was drawn from U(5, 25) mL. Rank agreement with the base case was measured by Spearman correlation. All calculations used Python 3 with NumPy and SciPy, and figures were produced with Matplotlib. Scripts, input tables and a provenance record of every computed value are supplied as supplementary material.

3. Results

3.1. Validation of the AGREE implementation

Recomputed from the published inputs, the three AGREE case studies gave 0.431, 0.371 and 0.249, against 0.43, 0.37 and 0.23 in the original report [17]. The first two agree to the reported precision. The Soxhlet case differs by 0.02, most likely because of an input detail not stated in the text. The implementation was therefore judged suitable for comparing methods.

3.2. Greenness and practicality of published methods

The risk analysis and AQbD workflow are summarised in Figure 1, and the criterion-level AGREE profile in Figure 2a. Overall AGREE scores lay in a narrow, low band from 0.39 (M2) to 0.50 (M7), with a median of 0.45 (Table 2). No method reached 0.60. Six criteria contributed the same value to every method: principles 1, 2, 3, 4, 5 and 6 depend on the off-line, manual tablet preparation that all ten share. The differences therefore came from waste (0.21-0.25), throughput (0.53-0.85), energy, renewable reagents, toxic reagent volume (0.04-0.23) and operator hazards (0.40-0.80). Toxic reagent volume was the weakest criterion throughout. Methanol-water systems (M7, M10) gained on principle 10, and the two ultra-high-performance methods (M1, M5) gained on principle 9. The acetonitrile method acidified with perchloric acid (M2) scored lowest because it carries three hazard classes and no renewable reagent.

Table 2. Computed greenness, practicality and retention indicators for the published methods and the two design targets.

ID	Run time (min)	Mobile phase per run (mL)	Toxic reagent per sample (mL)	AGREE (base)	AGREE, Monte Carlo 95% interval	BAGI	Estimated k1
M1	5.64*	2.26	26.13	0.49	0.42-0.54	82.5	3.09
M2	4.92*	4.92	11.97	0.39	0.31-0.43	82.5	-0.24
M3	3.31*	3.31	11.32	0.41	0.34-0.45	82.5	-0.21
M4	6.0	10.2	28.32	0.43	0.37-0.47	80.0	1.28
M5	2.94*	4.41	10.29	0.47	0.40-0.52	82.5	NR
M6	6.01*	6.01	13.95	0.44	0.37-0.48	80.0	0.00
M7	5.0	2.5	16.5	0.50	0.43-0.54	82.5	1.93
M8	4.0	6.0	17.3	0.46	0.39-0.50	82.5	0.03
M9	10.0	10.0	35.0	0.43	0.37-0.47	80.0	0.07
M10	11.0*	13.2	30.56	0.47	0.40-0.52	80.0	0.19
G1	8.0	8.0	0	0.52	0.47-0.57	80.0	2 (design)
G2	3.0	1.2	0	0.59	0.53-0.64	82.5	2 (design)

*Run time not reported; 1.2 x retention time of the last peak. k1, retention factor of the first-eluting drug at porosity 0.65; NR, column dimensions not reported.

BAGI discriminated even less. All ten methods scored 80.0 or 82.5, and the only attribute that varied was throughput, which depends on whether the run was shorter than 6 min. The two indices were not correlated (Spearman rho 0.18, p = 0.621). The Monte Carlo analysis showed that the AGREE ranking was stable to the coding rules (median Spearman correlation with the base ranking 0.98, 5th percentile 0.93). M7 was the greenest published method in 68.6% of draws and M1 in 29.9% (Figure 2b).

3.3. Retention adequacy

The retention screen gave a different picture from the greenness scores (Figure 3). Of the nine methods with reported column dimensions, only M1 gave an estimated k1 of at least 2 (3.09). M7 (1.93) and M4 (1.28) were close, and six methods gave k1 below 0.5. For the 250 x 4.6 mm column at 1.0 mL/min the estimated dead time is 2.70 min. The reported retention times of empagliflozin in M2 (2.05 min) and M3 (2.139 min) are shorter than this, which gives negative retention factors that no plausible porosity removes (-0.10 to -0.30 for M2). Elution order also varied. Linagliptin eluted first in M1 and M4, and empagliflozin eluted first in the other eight methods, with no simple relationship to buffer pH (M5, buffered at pH 4, eluted empagliflozin first). Where both k values were positive and k1 was at least 1, selectivity lay between 2.18 and 2.66.

Extending run times to meet the k_last = 3 condition cost little in greenness. AGREE fell by at most 0.03 (M2 0.36, M3 0.38, M6 0.42, M8 0.44, M9 0.42). BAGI fell to 77.5 for the four methods whose run time exceeded 12 min.

3.4. Risk ranking

Two failure modes exceeded the high-risk threshold (Figure 1b): mobile-phase pH (RPN 60) and organic modifier type and fraction (RPN 40). Sonication and filtration (27), column chemistry and dimensions (24), column temperature (18) and sample diluent and volume (18) formed a middle tier. Flow rate (9),

detection wavelength (6) and injection volume (4) were ranked low, since each is readily controlled by the instrument and monitored through system suitability.

3.5. Greenness design space

Under the retention constraint, the minimum mobile-phase consumption per run depends only on column volume: 12.96 mL for 250 x 4.6 mm, 7.78 mL for 150 x 4.6 mm and 1.08 mL for 100 x 2.1 mm, whatever the flow rate. On the feasible boundary, AGREE therefore improved as run time shortened, through throughput, and as column volume fell, through waste and energy class (Figure 4). With ethanol and phosphate, the highest feasible AGREE was 0.52, 0.54 and 0.60 for the three formats. The corresponding values were 0.46, 0.48 and 0.55 with methanol and 0.40, 0.42 and 0.49 with acidified acetonitrile. At the G1 operating point the modifier alone moved AGREE from 0.41 with acetonitrile through 0.46 with methanol to 0.52 with ethanol. Even if the six chromatography-dependent criteria all scored 1, the fixed tablet-preparation criteria would cap AGREE at 0.71.

Design target G1 (150 x 4.6 mm, 1.0 mL/min, 8.0 min) used 8.0 mL of mobile phase per run and gave AGREE 0.52 and BAGI 80.0. Design target G2 (100 x 2.1 mm, 0.4 mL/min, 3.0 min) used 1.2 mL per run, generated 11.4 mL of total waste per sample and gave AGREE 0.59 and BAGI 82.5. Increasing the preparation volume from 10 to 25 mL lowered G2 only to 0.58. Across the simulations, the less green of the two targets exceeded every published method in 92.9% of draws, and G2 reached AGREE 0.60 or more in 31.4% of draws.

4. Discussion

The central finding is that the published methods cluster in a narrow greenness band, and that this band is set more by what the methods share than by what distinguishes them. Every method requires off-line, manual tablet extraction, and that alone fixes half of the AGREE clock. The structural ceiling of 0.71 is a useful reference for this reason: it shows that a chromatographer optimising only the separation can never pass that value for a tablet assay, and that real gains beyond it must come from preparation (smaller volumes, fewer steps, automated or in-vial extraction) rather than from the column. The AGREE authors noted that the tool is designed to reward such structural choices [17], and the present data show how strongly they weigh in a routine assay.

Within the band, greenness was decided by modifier toxicity, the volume of solvent per run and platform energy. Methanol-water methods scored best because they avoid acid hazards and use a potentially renewable solvent. Solvent selection guides point in the same direction [27,28]. The toxic-reagent criterion gave the lowest scores of all, and it is also the easiest to change. Replacing methanol or acetonitrile with ethanol removes the toxic volume entirely, and at the G1 operating point this substitution alone was worth more than any plausible change of flow rate. This agrees with the wider green

chromatography literature, which places solvent choice ahead of instrument settings [24,25,29].

The retention screen adds an analytical dimension that greenness metrics do not capture. A retention factor below 1 means that an analyte spends less time in the stationary phase than in the mobile phase. Selectivity then depends heavily on extra-column effects, and co-elution with excipients or degradation products at the solvent front becomes likely. The FDA reviewer guidance sets k above 2 for this reason [38]. The finding that six of nine methods fall far below this value, and that two report retention times shorter than the estimated dead time of their stated column, is best read as a warning about reporting rather than as proof of failure. Column dimensions may have been misreported, flow rates may differ from those stated, or retention times may have been read from an unusual time origin. Whatever the cause, a stability-indicating claim is hard to sustain for a peak with k near zero. This matters for greenness assessment too: a short run bought with inadequate retention inflates the throughput criterion. The rescoring showed the inflation was small here (at most 0.03), so the ranking holds, but the principle is worth stating. Greenness should be scored only after analytical adequacy is established, which is the White Analytical Chemistry position [18].

The disagreement between AGREE and BAGI deserves comment. BAGI was designed to separate methods that differ in instrumentation, preparation complexity and throughput [19]. For a set of reversed-phase UV assays with similar tablet preparation, nine of the ten attributes are identical and the tenth changes by one level at a 6 min run time. BAGI values of 80-82.5 confirm that these methods are practical by any standard, but the index cannot rank them. Reporting AGREE and BAGI together, as recent studies do [20-23], is still useful: the pair shows that the choice among these methods is a greenness decision, not a practicality one.

The AQbD elements explain why the design targets differ from the published methods. The two high-risk factors, pH and modifier, are exactly those that control linagliptin retention. With a pKa of 8.6 [4], linagliptin is fully protonated across the usual reversed-phase pH range, and its retention and peak shape depend on silanol activity and ion pairing with buffer or acid anions. This is consistent with the reversal of elution order between methods seen in Table 1, which tracks column, modifier and counter-ion together rather than pH alone. Empagliflozin, which is neutral, responds mainly to organic strength. A design that fixes pH with a phosphate buffer and uses modifier fraction as the main tuning variable therefore separates the two effects, which is the purpose of a risk-based design. Ethanol's higher eluotropic strength relative to methanol [29] suggests that a lower organic fraction than in the methanol methods will be needed, and phosphate buffer avoids the corrosive or oxidising acids that lowered principle 12 for M2-M4 and M6. Column temperature entered the middle risk tier because ethanol-water mixtures are viscous [29]. Moderate heating is the obvious control, and the harmonised USP chapter on chromatography

allows adjustments of column temperature and of column length relative to particle size [45].

The design space itself carries a practical message. Because minimum mobile-phase use is fixed by column volume, lowering flow rate on a large column saves no solvent once retention is adequate; it only lengthens the run. Solvent use falls only when column volume falls. G2, on a 100 x 2.1 mm column, used 1.2 mL of mobile phase per run against 8.0 mL for G1 and a constrained minimum of 12.96 mL for the 250 x 4.6 mm column that most published methods used. Laboratories without ultra-high-performance equipment can still follow G1, which scored higher than every published method in almost every simulation while keeping the column format most laboratories already hold.

The limitations are specific and should be stated plainly. First, all scores rest on coding rules applied to reports that describe sample preparation unevenly. The Monte Carlo analysis showed the ranking to be robust (median rank correlation 0.98), but absolute AGREE values shift within the intervals in Table 2 as preparation volume and weights change. Second, the retention factors are estimates from nominal geometry and assumed porosity, not measured dead times, and they are only as reliable as the reported column dimensions. Third, the FMEA scores are expert judgements. Fourth, and most important, G1 and G2 are design targets, not validated methods. Their greenness scores are computed for operating conditions that satisfy a retention constraint in theory. Whether ethanol-phosphate on a C18 phase actually resolves empagliflozin, linagliptin and their degradation products with k1 of at least 2, resolution of at least 2 and acceptable tailing has not been tested. No chromatogram, recovery or precision value is reported here, and none should be inferred. Finally, the literature set is limited to accessible, peer-reviewed reports. Several further methods could not be scored because their conditions were unavailable.

These limits point to a defined experimental next step. A face-centred or Box-Behnken design over ethanol fraction, buffer pH (for example 3.0-5.0) and column temperature on a 100 x 2.1 mm C18 column at 0.4 mL/min would test whether G2 is feasible. The responses should be k1, resolution, tailing and run time, and Monte Carlo propagation of model error should be used to define a probabilistic operable region [12,13]. Forced degradation would confirm specificity, and full ICH Q2(R2) validation [6] would follow. The AGREE and BAGI scores of the resulting method can then be compared directly with the values reported here.

5. Conclusion

An in silico AQbD analysis of ten published chromatographic methods for empagliflozin and linagliptin showed moderate greenness (AGREE 0.39-0.50), uniformly high practicality (BAGI 80-82.5) and, in most methods, retention too low to support confident specificity. Greenness in this assay is governed by column volume, preparation solvent and modifier toxicity, not by flow rate, and the manual tablet preparation caps

AGREE at 0.71. An ethanol-phosphate method on a narrow-bore column is predicted to reach AGREE 0.59 with adequate retention. That prediction is a hypothesis to be verified in the laboratory, and the design space defined here specifies where the verification should start.

Declarations

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Conflicts of interest. The authors declare no conflicts of interest.

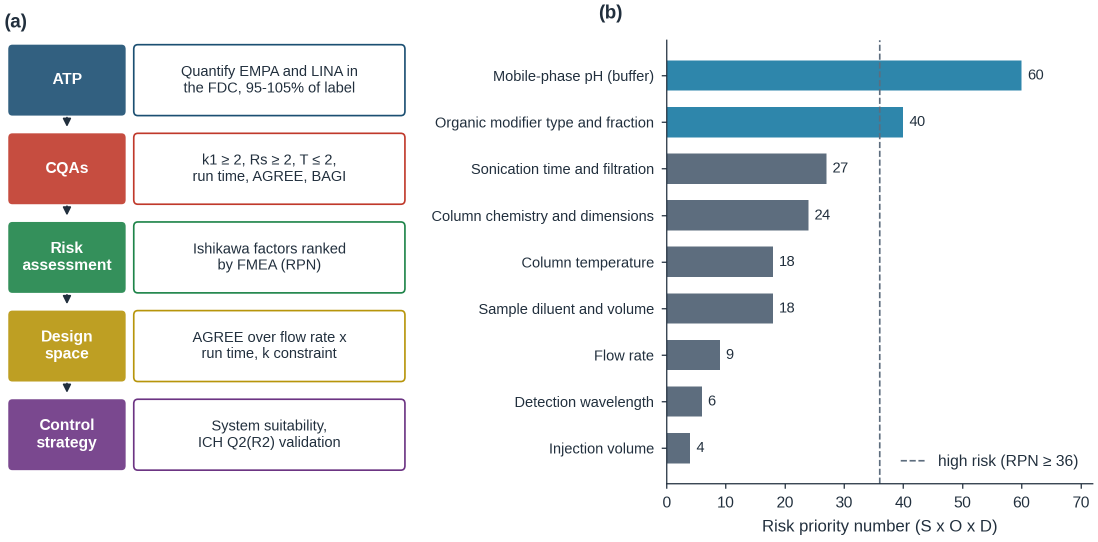

Figure 1. AQbD framework and risk ranking for the simultaneous assay of empagliflozin (EMPA) and linagliptin (LINA). (a) Workflow from analytical target profile (ATP) through critical quality attributes (CQAs), risk assessment and design space to control strategy. (b) Failure mode and effects analysis: risk priority number (RPN = severity x occurrence x detectability, each scored 1-5 by the authors) for nine method factors. The dashed line marks the high-risk threshold (RPN 36); bars above it are shaded teal. Scores are expert judgements.

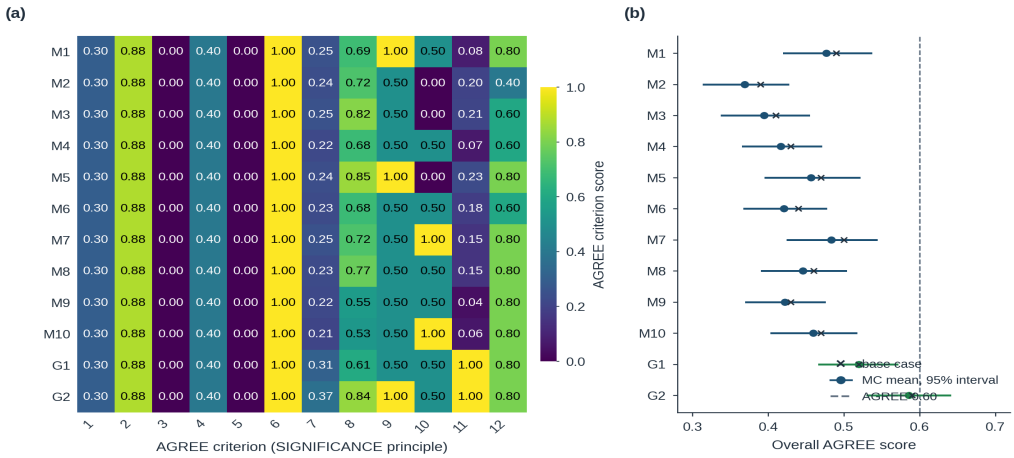

Figure 2. AGREE assessment of ten published methods (M1-M10) and two design targets (G1, G2). (a) Criterion scores (0-1) for the twelve SIGNIFICANCE principles under uniform coding rules; criteria 1-6 are identical across methods because all use off-line manual tablet preparation. (b) Overall AGREE score: crosses, base case; circles and bars, mean and 95% interval of 5000 Monte Carlo draws varying sample mass,

preparation volume, step count, imputed run time and criterion weights (Dirichlet, concentration 20). The dashed line marks 0.60.

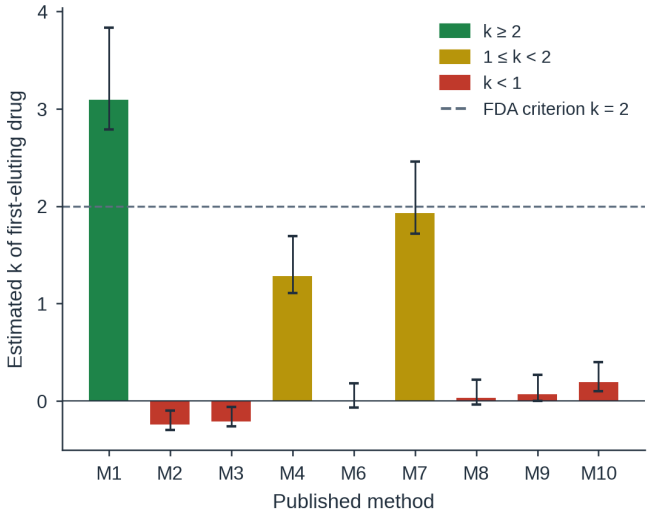

Figure 3. Estimated retention factor of the first-eluting drug (k1) for nine published methods with reported column dimensions. Bars use a total porosity of 0.65; whiskers span porosities of 0.55 and 0.70. The dashed line is the k = 2 criterion of the FDA reviewer guidance [38]. Negative values arise when the reported retention time is shorter than the estimated column dead time. M5 is omitted because its column dimensions were not reported.

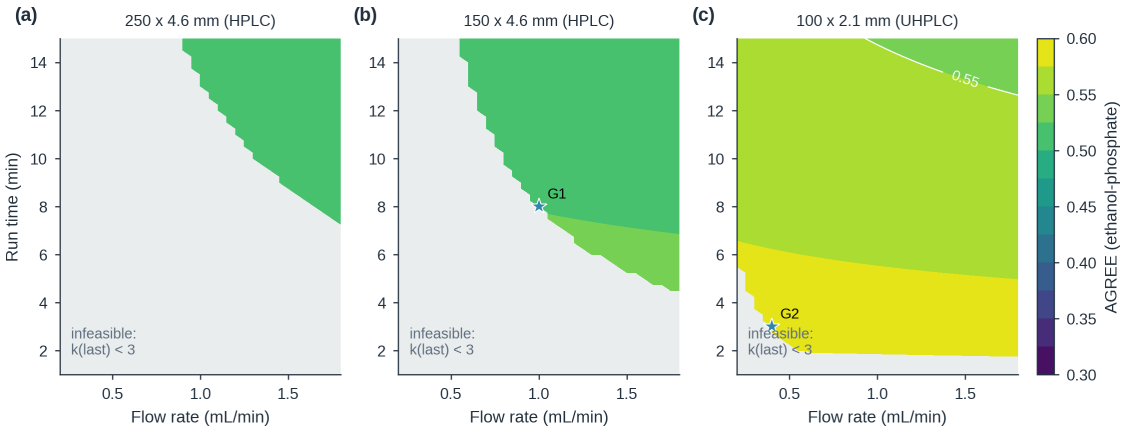

Figure 4. Greenness design space for an ethanol-phosphate mobile phase. AGREE is shown over flow rate and run time for (a) 250 x 4.6 mm, (b) 150 x 4.6 mm and (c) 100 x 2.1 mm columns, with 10 mL preparation solvent. Grey areas are infeasible because the last peak would elute with k below 3 (first peak k below 2 at selectivity 1.5). White contours mark AGREE 0.50 and 0.55. Stars mark design targets G1 (1.0 mL/min, 8.0 min) and G2 (0.4 mL/min, 3.0 min).

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