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Column selection considerations in HPLC method transfer for related substances of acetylsalicylic acid in tablets

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Abstract: *Analytical methods for the quality control of pharmaceutical raw materials and finished products are routinely transferred between laboratories, including contract research organizations and competent authority/official medicines control laboratories (OMCLs) equipped with diverse instrumentation. Successful method transfer is essential to ensure reproducible results, regulatory compliance, and consistent product quality, while minimizing the need for partial or full method re-validation. Reversed-phase high-performance liquid chromatography (RP-HPLC) remains the dominant separation technique, accounting for more than 90% of chromatographic applications. Despite the availability of over 600 commercial RP-HPLC columns, substantial differences in selectivity and manufacturing characteristics exist even among nominally similar stationary phases, and no single column is universally applicable. Consequently, rational selection of equivalent columns during method transfer is challenging, as column performance is influenced by multiple interaction mechanisms and physicochemical parameters. Although a number of column selection tools are available, truly experiment-free column equivalence assessment remains elusive.*

The presented examples of HPLC method transfer for determination of related substances of acetylsalicylic acid in two different tablet formulations illustrate that identifying suitable column alternatives is often non-trivial, inevitably requiring supplementary experimental work. This points to the opportunity for improved and simplified method transfer strategies and suggests that advanced technologies, such as artificial intelligence, may offer valuable support in column selection, thereby reducing experimental burden, time, and environmental impact.

Key Word: *RP-HPLC; Method transfer; Related substances; Acetylsalicylic acid; Column selection.*

I. Introduction

Analytical methods for the assessment of pharmaceutical raw materials and finished products are routinely transferred between laboratories, including contract research organizations and competent authority/official medicines control laboratories (OMCLs) equipped with different instrumentation. Successful method transfer is essential to ensure reproducible results, regulatory compliance, and consistent product quality, regardless of the laboratory or equipment used, while preferably minimizing the need for partial or full re-validation. The process involves a fully documented demonstration that the receiving laboratory is capable of performing the method reliably, with any observed deviations appropriately addressed through partial or complete re-validation, when necessary[1,2]. Effective method transfer not only prevents the release of products that fail to meet quality specifications but also avoids the unwarranted rejection of compliant medicinal products.

Reversed-phase high-performance liquid chromatography (RP-HPLC) remains the predominant chromatographic technique, representing over 90% of separations. Although more than 600 RP-HPLC columns are commercially available, significant differences in selectivity and manufacturing quality exist even for apparently similar columns, and no single column is universally suitable for all analytes[3]. As a result, analytical laboratories often maintain a diverse inventory of columns, but given the extensive variety of chromatographic columns available on the market, rarely any laboratory could afford to have access to the specific column recommended in the method, in all situations. Rational selection of equivalent columns is challenging due to multiple factors influencing column behavior, including primary dispersive (London) interactions, polar, hydrogen bonding, and electron pair donor–acceptor interactions. Additional variables such as packing type, residual silanol content, end-capping, ligand bonding density, and pore size further contribute to column-specific

characteristics[3,4]. These factors highlight the importance of careful column selection to achieve reproducible and robust separations. Accordingly, column choice represents a critical step in RP-HPLC method transfer, even for the analysis of seemingly simple samples. Although a number of tools have been developed to support the selection of equivalent HPLC columns, a truly "experiment-free" approach to column equivalence assessment remains largely unavailable.

In this study, practical examples of the selection of equivalent HPLC columns are presented to demonstrate successful method transfer, without the need for partial or full re-validation, for two RP-HPLC related substances methods of acetylsalicylic acid applied to two different tablet formulations.

II. Material And Methods

Reagents and chemicals: Working standards of acetylsalicylic acid and salicylic acid generously provided by ExtractumPharma, Hungary, were used for method transfer in Example 1. Certified reference material of acetylsalicylic acid obtained from Sigma-Aldrich and certified reference material of salicylic acid obtained from Supelco were used for method transfer in Example 2. Tested medicinal products were obtained from a local pharmacy. Formulation 1 were tablets containing the active substances acetylsalicylic acid and caffeine, with a declared content of 500 mg and 50 mg per tablet, respectively. Formulation 2 were gastro-resistant tablets containing the active substance acetylsalicylic acid, with a declared content of 100 mg. Acetonitrile (HPLC isocratic grade), acetonitrile (HPLC gradient grade) and methanol (HPLC gradient grade) were purchased from Carlo Erba Reagents, Val de Reuil, France. Potassium dihydrogen phosphate (for analysis) and o-phosphoric acid 85% HPLC were purchased from Merck, Darmstad, Germany. Water for chromatography was obtained with a TKA-LAB Reinstwasser system (Niederelbert, Germany).

Chromatographic Conditions: In Example 1 the method transfer was performed on a Shimadzu UHPLC Nexera System (equipped with a LC-30AD binary pump, DGU-20A5 on-line degasser, SIL-30AC autosampler, CTO-20AC column oven and SPD-M20A PDA detector), initially using a Purospher STAR RP-18e, 250 x 4.6 mm, 5 µm, chromatographic column (Merck), and afterwards finalized on an XBridge C18, 250 x 4.6 mm, 5 µm, column (Waters Corporation). Data were acquired and processed by use of LabSolutions LC/GC software (Shimadzu Corporation). In Example 2 the method transfer was conducted on an Agilent HPLC 1200 Series system (equipped with a G1312B binary pump, G1379B micro vacuum degasser, G1367C autosampler, G1316B column compartment and G1315C PDA detector), using a Symmetry C18, 250 x 4.6 mm, 5 µm, chromatographic column (Waters Corporation). The Chem Station for LC3D software (Agilent Technologies) was used for instrument control, data acquisition and processing.

III. Results and discussion

The results presented in this section illustrate practical aspects of HPLC method transfer, with a particular focus on the selection of equivalent chromatographic columns. Method transfer performance was evaluated primarily through compliance with predefined system suitability criteria, as specified in the originating methods and in accordance with the requirements of Ph. Eur. 2.2.46[5]. Successful transfer was defined as the achievement of acceptable chromatographic performance without the need for modifications beyond the permitted limits and without initiating partial or full method re-validation.

Two representative examples are discussed, involving RP-HPLC methods for the determination of related substances of acetylsalicylic acid in different tablet formulations. The examples were selected to reflect common challenges encountered in routine laboratory practice, including differences in column chemistry, elution mode (gradient versus isocratic), and the limited predictive power of available column selection tools. The impact of column choice on chromatographic selectivity, resolution, peak symmetry, and overall system suitability is discussed for each case.

Example 1: Method transfer for determination of related substances of acetylsalicylic acid in acetylsalicylic acid/caffeine tablets, using RP-HPLC method with gradient elution

The originating laboratory method for related substances of acetylsalicylic acid represents a gradient elution RP-HPLC method, using a mobile phase consisting of solvent A and solvent B (respective mixtures of o-phosphoric acid 85%, acetonitrile and methanol), that specifies a Reprosil-Pur ODS-3 chromatographic column (250 × 4.6 mm, 5 µm), with detection at 237 nm. At our laboratory, method transfer was initiated on a Purospher STAR RP-18e (250 × 4.6 mm, 5 µm). The choice of the column was based on the stationary phase properties and analytical experience, since no other data could be found in available column selection tools. To achieve system suitability, suitable adjustments of different method parameters were made, in line with the permitted adjustments of the chromatographic conditions for gradient elution (Ph.Eur. 2.2.46). After all allowable method adjustments were exhausted and system suitability wasn`t obtained, in order to mitigate the need for partial or full re-validation, method transfer was continued on another column with theoretically similar characteristics of the stationary phase.

An XBridge C18 (250 × 4.6 mm, 5 µm) was chosen. In this case no further adjustments of the parameters in the originating method were required in order to achieve system suitability. Specified requirements and results obtained for system suitability parameters regarding selectivity are given in Table no 1.

Table no 1: Specified requirements and obtained results for system suitability parameters during method transfer for determination of related substances of acetylsalicylic acid in acetylsalicylic acid/caffeine tablets, using RP-HPLC method with gradient elution.

	t_R / min	r_G	R_s	A_s	N / theoretical plates per column
Specified requirements for system suitability parameters	Given for information	Given for information	min 8.0	0.8 -1.5	min 50 000
Acetylsalicylic acid (ASA)	11.333	1.00	/	1.1	121 003
Salicylic acid (SA)	12.959	1.14	11.8	1.3	127 823

t_R - retention time; r_G - relative retention, unadjusted; R_s - resolution; A_s - symmetry factor; N - number of theoretical plates (parameter results expressed according to Ph.Eur. 2.2.46)

Example 2: Method transfer for determination of related substances of acetylsalicylic acid in acetylsalicylic acid gastro-resistant tablets, using RP-HPLC method with isocratic elution

For determining related substances of acetylsalicylic acid, an isocratic RP-HPLC method was proposed at the originating laboratory, using a mobile phase consisting of a mixture of a potassium dihydrogen phosphate buffer and acetonitrile, on a Luna C18 chromatographic column (250 × 4.6 mm, 5 µm), with detection at 280 nm. To assist with the choice of an equivalent column available at the lab, the publicly available USP Column Equivalency Application[6] was used, indicating that the portfolio of HPLC columns didn`t contain any columns that were most likely to give an equivalent and acceptable separation. The closest equivalent column available in the lab was Symmetry C18 250 × 4.6 mm, 5 µm, having column similarity factor, F = 3.83. According to the column selection tool criteria, HPLC columns with values of F > 3, may or may not provide a similar or adequate separation, depending on the complexity of the separation. In this case, the method transfer was successful without any requirement for adjustment of the originating laboratory method. Specified requirements and results obtained for system suitability parameters regarding selectivity are presented in Table no 2. Interestingly enough, although the column selection tool didn`t cite the particular column as one of the most likely to give an equivalent separation, obtained system suitability results were superior to the results presented in the validation of the originating laboratory method, demonstrating that Symmetry C18 can provide better separation for the specific application, than the original column.

Table no 2: Specified requirements and obtained results for system suitability parameters during method transfer for determination of related substances of acetylsalicylic acid in acetylsalicylic acid gastro-resistant tablets, using RP-HPLC method with isocratic elution.

	t_R / min	r_G	R_s	A_s	N / theoretical plates per column
Specified requirements for system suitability parameters	Given for information	Given for information	min 1.5	0.8 -1.5	min 2000
Acetylsalicylic acid (ASA)	9.984	1.00	/	0.9	7440
Salicylic acid (SA)	16.626	1.67	11.4	1.5	8967

t_R - retention time; r_G - relative retention, unadjusted; R_s - resolution; A_s - symmetry factor; N - number of theoretical plates (parameter results expressed according to Ph.Eur. 2.2.46)

IV. Conclusion

The presented examples of HPLC method transfer reflect routine practice in the receiving laboratory and illustrate that, despite the availability of modern column selection tools and established theoretical knowledge, the identification of a truly equivalent chromatographic column remains a non-trivial task. This difficulty is largely attributable to the extensive diversity of commercially available stationary phases and their numerous chemical modifications.

Adjustments of chromatographic conditions beyond established allowable limits in order to achieve comparable or acceptable separation performance necessitate partial or complete method re-validation. Such re-validation is associated with increased experimental workload, extended analysis timelines, and higher consumption of laboratory resources. Consequently, it is often more efficient to invest additional effort at an early stage in identifying a suitable column alternative rather than proceeding directly to method re-validation.

In routine analytical laboratory practice, method transfer therefore remains a demanding and time-consuming process that inevitably requires supplementary experimental work. The present study indicates that there is still scope for improvement and simplification of method transfer workflows. In particular, greater utilization of advanced technologies, such as artificial intelligence, may offer valuable support in the critical step of column selection, which fundamentally governs chromatographic separation. Such developments have the potential to further reduce time and resource consumption and, ultimately, contribute to a lower environmental impact.

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