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Is supercritical fluid chromatography hyphenated to mass spectrometry suitable for the quality control of vitamin D3 oily formulations?☆

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ABSTRACT

Nowadays, many efforts are devoted to improve analytical methods regarding efficiency, analysis time and greenness. In this context, Supercritical Fluid Chromatography (SFC) is often regarded as a good alternative over Normal Phase Liquid Chromatography (NPLC). Indeed, modern SFC separations are fast, efficient with suitable quantitative performances. Moreover, the hyphenation of SFC to mass spectrometry (MS) provides additional gains in specificity and sensitivity. The present work aims at the determination of vitamin D3 by SFC-MS for routine Quality Control (QC) of medicines specifically. Based on the chromatographic parameters previously defined in SFC-UV by Design of Experiments (DoE) and Design Space methodology, the method was adapted to work under isopycnic conditions ensuring a baseline separation of the compounds. Afterwards, the response provided by the MS detector was optimized by means of DoE methodology associated to desirability functions. Using these optimal MS parameters, quantitative performances of the SFC-MS method were challenged by means of total error approach method validation. The resulting accuracy profile demonstrated the full validity of the SFC-MS method. It was indeed possible to meet the specification established by the European Medicines Agency (EMA) (i.e. 95.0 – 105.0% of the API content) for a dosing range corresponding to at least 70.0-130.0% of the API content. These results highlight the possibility to use SFC-MS for the QC of medicine and obviously support the switch to greener analytical methods.

1. Introduction

Supercritical fluid chromatography (SFC) is nowadays expanding in the field of separation sciences [1]. Indeed, the combination of the modern robust instruments and the typical mobile phase of SFC allows fast, effective green analysis, which benefits to many domains and especially the pharmaceutical field [2]. Moreover, the technique is also compatible with the most popular detectors employed in chromatography [3]. So, to specifically detect compounds in complex samples with potential matrix interferences, it is therefore possible to hyphenate SFC to mass spectrometry (MS), which provides new levels of specificity and sensitivity [4–6]. Many application of SFC-MS are currently described for the detection of compounds (e.g. drugs, metabolites, lipids. . .) in challenging matrices having various origins (biological fluid, vegetal extract, . . .) [7–20].

Vitamin D3 (cholecalciferol) is a fat-soluble vitamin having plethora of effects, which are divided in two classes: skeletal and extra-skeletal effects [21,22]. The skeletal effects being in favour to the bone health, while the extra-skeletal effects are related to its beneficial action to e.g. the immune system, the cognition, the cardiovascular system, etc. All these effects are still under fruitful investigation, which explains why the vitamin D3 is the most published vitamin of the century [23]. However, despite these benefits, it appears that the large majority of the population has a deficiency in vitamin D3 [24]. To address this issue, an exogenous supply of vitamin D3 (i.e. by medicines) is usually prescribed to patients [21].

To ensure the quality and safety of the produced medicine, a quality control (QC) is a mandatory step which is performed according to the specifications established by the European Medicines Agency (EMA). Thus, the content of the active pharmaceutical ingredient (API) must be comprised within a ± 5.0% interval centred on the declared content of the drug (i.e. 95.0–105.0%) [25]. In this

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context, the quantitative performances of the analytical method should be properly established by means of method validation, demonstrating that the method is fit for its intended purpose.

For the analysis of vitamin D3, normal phase liquid chromatography (NPLC) is often employed due to the highly hydrophobic nature of this compound [26–30]. However, SFC is frequently suggested as an alternative to NPLC analysis [31]. Various study reports the use of SFC for the determination of vitamin D3 [32–35] but most of these methods are non- or only partially validated [32–34].

With the current expansion of both SFC and MS in the field of separation sciences, the objective of the present work was to evaluate the quantitative performances of SFC-MS method for pharmaceutical quality control. In this context, the more challenging determination of vitamin D3 in a medicine (i.e. complex oily formulation for which ±5.0% specifications apply) was selected as a relevant case study. So, with an assessment of potential matrix effect, the QC of dietary supplements (wider specifications: e.g. −20.0, 50.0%) would be feasible. In a previous project, an analytical SFC-UV method was optimized to separate vitamin D3 and its related impurities [35]. However, the optimization of the hyphenation to MS detector and responses were still required. Also, as this SFC-MS method should be used in routine laboratories, this implementation should be simple and reliable. Consequently, a relatively simple MS device was employed, which consisted of a robust and compact single quadrupole MS detector with ESI ionization source (see Section 2.1 SFC-MS instrumentation). Moreover, only the adjustment of few parameters (i.e. cone voltage, capillary voltage…) was possible for the user, which obviously speed up the tuning and ease the routine use of MS detection. Given the importance of the make-up solvent in the ionization process and its final impact on the MS signal response, this work started with empirical testing of various classic make-up solvents mixtures. This screening permitted to highlight a preferential make-up solvent composition. Afterward, the Design of Experiments (DoE) methodology was employed to efficiently optimize make-up composition and MS detector parameters. The targeted goal was to increase method sensitivity, which was expressed as the maximization of the signal to noise ratio (S/N) through a desirability function. Once MS detection was optimized, the quantitative capabilities of SFC-MS were challenged by means of a full analytical method validation [36]. This was performed with a total-error approach using ß-expectation tolerance interval and accuracy profiles as a drastic decision tool [37–40]. This methodology permitted to control and appreciate the level of risk linked to future routine application of the method [41,42].

This study constitutes a further step in our work devoted to the implementation of the SFC for pharmaceutical QC [35,43]. To the best of our knowledge, among all the SFC-MS method available in the literature, the present paper reported in literature a first fully validated SFC-MS method for QC of medicines.

2. Material and method

2.1. SFC-MS instrumentation

A Waters Acquity UPC^{2TM} system (Waters Corp., Milford, USA) was used to carry out the experiments. Compared to previous description of this system [35], the PDA detector was shunt and a dedicated interface (Waters Isocratic Solvent Manager − ISM) was employed for the hyphenation to a Waters Acquity QDa mass detector (performance version). The ISM interface module comprised a SFC dedicated flow splitter (SFC ratio = 1/5) and an isocratic pump, which ensured the post-column make-up solvent addition (flow rate = 0.5 mL min^{-1}).

The analytical column employed in this study was an Acquity UPC^2 BEH column (3.0 × 100 mm; 1.7 μm) provided by Waters.

2.2. Chemicals and reagents

Cholecalciferol (vitamin D3; 99.0%) and Ergocalciferol (vitamin D2; 99.2%) were purchased from Sigma Aldrich (St Louis, MO, USA). 5,6-trans-cholecalciferol (95%) and pre-vitamin D3 (>80%) were sourced from TRC Inc. (Toronto, Canada). Batches of oily matrix of the medicine (i.e. not containing any API; confidential composition) were produced and used to reconstitute the various samples required in this study.

Ethanol (HPLC gradient grade) and ammonium acetate (AA; >98.0%) were supplied by Merck Millipore (Darmstadt, Germany). Ammonium formate (AF; 98.7%) was bought from VWR chemicals (Leuven, Belgium). Methanol and *n*-heptane (HPLC grades) were purchased from J.T. Baker (Deventer, Netherlands). Water (MS grade) and 2-propanol (MS grade) were sourced from Biosolve BV (Valkenswaard, Netherlands). Carbon dioxide grade 4.5 (99.995%) was obtained from Westfalen BVBA (Aalst, Belgium).

2.3. Analytical method

2.3.1. SFC method

SFC method for the separation of vitamin D3 and its related impurities was previously optimized using DoE-DS methodology [35]. Briefly, this method employed ethanol as co-solvent with gradient mode elution on a hybrid bare silica column (Acquity UPC^2 BEH: 3.0 × 100 mm; 1.7 μm). Validated optimal conditions included a mobile phase flow rate equal to 2.0 mL min^{-1}, 2.0 μL of sample injected, active back pressure regulator (BPR) set to 110 bar and heating of the column to 41 °C. In these conditions and with a mobile phase composed of CO_2 and ethanol (98:2 v/v), the pressure recorded at the beginning of the run was equivalent to 230 bar (head of the pump).

2.3.2. Make-up solvent screening

Various make-up solvents were tested and the mean signal intensity was monitored (ESI, positive mode, single ion record (SIR)). Experiments consisted in triplicate injections of vitamin D3 (m/z = 385.48; 2.0 μL injected; 1 μg mL^{-1} in pure *n*-heptane), under given SFC conditions. This allowed to directly consider potential effects of the carbon dioxide and organic modifier in actual analytical conditions. The make-up solvents tested were composed of i) methanol + ammonium formate (0.06% m/v), ii) methanol + ammonium formate (0.1% m/v), iii) methanol + ammonium formate (0.2% m/v), iv) methanol + ammonium formate (0.4% m/v), v) methanol: H_2O (9:1) + ammonium formate (0.2% m/v), vi) ethanol + ammonium formate (0.2% m/v), vii) methanol + ammonium acetate (0.2% m/v).

For this screening, MS parameters were set as following: cone voltage (25 V), capillary voltage (1.5 kV), probe temperature (600 °C) and acquisition frequency (8 pt sec^{-1}). All make-up solvents were added post-column at a flow rate fixed to 0.5 mL min^{-1}; split ratio being constant and equal to 1/5.

2.3.3. MS optimization: design and computation

The make-up screening allowed selecting a make-up composition, the second step was to optimize MS parameters to improve method sensitivity. Thus, a four factors Central Composite Design (CCD) involving triplicate of the central point, as described in Table 1, was employed.