Optimization of an HPLC-DAD Method for Quercetin Detection

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Abstract. *High-performance liquid chromatography (HPLC) is one of the most used liquid chromatographic techniques owing to its versatility that allows an optimal resolution. Quercetin (QUE), a polyphenol, has received increasing attention due to its antioxidant properties. Efforts have been made to overcome the traditional poor oral bioavailability of quercetin, requiring the development of analytical techniques for quercetin detection in different matrices. The main objective of this work was the optimization of a HPLC-Diode Array Detector (DAD) method for quercetin. An optimization process was conducted to guarantee the best conditions and results. Detection and quantification were executed with a reverse phase C-18 column. Different mobile phase (MP) compositions, flow rates, and wavelengths (with DAD) were studied. Better results were achieved with a MP composition of water:acetonitrile:methanol (55:40:5), with 1.5% acetic acid at a 1.3 mL/minute flow rate, with good results regarding chromatogram resolution, and quercetin quantification and detection.*

Keywords. High-performance liquid chromatography, HPLC, quercetin, optimization, tailing

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1 Introduction

Chromatography, a powerful analytical technique that allows the detection of compounds, can be applied for different pharmaceutical purposes [1]. This technique has the advantage that can be used to separate, purify, quantify and/or identify compounds in mixtures [1], [2].

Within the chromatographic techniques, the most widely used is HPLC, since this is a technique that allows qualitative and/or quantitative analysis [1], [2]. This technique consists of a MP containing the analyte of interest that is eluted at high pressure along a chromatographic column, where the stationary phase is contained [1], [2].

QUE is a phenolic compound with strong antioxidant properties and has received great attention in recent years from the scientific community. It has been suggested that QUE might become useful in the treatment of several diseases such as cardiovascular, skin, and neurodegenerative diseases, or cancer [3]. The low bioavailability of QUE might compromise its application at the industry level. Accordingly, several attempts have been made, to develop methodologies that improve the actual applicability of QUE [4].

The development of new methodologies to produce and/or administer QUE, implies the development of analytical techniques that allow the inspection of the newly developed enabling the detection of QUE, in different matrices.

The main objective of this work was the optimization of a HPLC-DAD for QUE detection.

2 Methodology

The present work was based on a method described in the literature by Zu *et al.* (2006) [5]. The analysis by HPLC were performed in a JASCO® equipment, coupled to a DAD detector (MD-4010) (which allows an analysis within a range of wavelengths for subsequent selection of the one with the best detection signal), with an autosampler (AS-4050), and a pump (PU-4180) [2]. A C-18 reversed phase column, (LiChroCART® 250-4; LiChrosorb®, RP-18, 250 mm x 4 mm, 5µm) was used as stationary phase. QUE standard solutions were prepared in water:acetonitrile:methanol (45:15:40) from a 0.49 mg/mL QUE solution in the same solvent, previously prepared with a QUE standard (≥ 95.0%; Sigma-Aldrich® Co., St. Louis, MO, United States of America). Different MP compositions were evaluated (Table 1), assessing different solvent rations and acid concentration, as well as different flow rates and wavelengths (254 nm and 368 nm). The analysis took place over a 10-minute period, with a 10 µL injection volume.

Table 1. Chromatographic condition evaluated for method optimization.

Condition		Analysis															
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**
C (%)	W	45	45	45	40	50	55	55	58	60	70	55	55	55	55	55	55
	A	15	15	15	15	35	40	40	40	40	30	35	24	25	40	40	40
	M	40	40	40	40	15	5	5	2	0	0	10	20	20	5	5	5
	Aa	0	0.5	1.0	1.0	1.0	1.0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Flow Rate (mL/min)		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.2	1.3	1.3	1.5

C: composition; W: water; A: acetonitrile; M: methanol; Aa: acetic acid.

To assess the method's reliability, it was evaluated for linearity, sensitivity, precision, accuracy, selectivity, and stability criteria, as recommended by the International Conference on Harmonization (ICH) [6].

3 Results and Discussion

The analysis A, mentioned in the Table 1, corresponded to those described in the study of Zu *et al.* (2006) that served as a starting point [5]. An improvement in the retention time of QUE was achieved, which resulted in a decrease in the analysis time and in the solvents consumption. However, the tailing phenomenon was quite evident, motivating the evaluation of different chromatographic conditions.

The tailing may be related to an overloading of the column, or with a high concentration of the analyte, so different techniques can be applied for its correction or elimination. Changes in the MP aiming at improving the affinity of the analyte; or the acidification of the MP, are two previously described strategies [2], [7].

By testing the proposed conditions, different results, that presented both advantages and disadvantages at the analysis level, were observed. In some situations, a decrease in tailing was verified, but, on the other hand, there was a significant increase in the retention time. Also, depending on the composition of the MP, different signal intensities were verified, which also influenced the future quantification of the compound under analysis, and that could eventually lead to a decreased sensitivity. Changes in the proportion of each of the three solvents of the MP were tested, with an increase in the percentage of aqueous phase, as well as an increase concentration of acid in the final solvent mixture. The flow rate at which the analysis was performed was also changed to understand if a variation in flow rate at the time of QUE detection had any impact on the resolution of the chromatographic peak.

After all the optimization process, analysis O proved to be the one that best corrected the tailing observed in the chromatographic peak of QUE, as well as decreased the retention time, allowing a reduction in the analysis time, and a lower solvents consumption.

Hence, a 50:40:5 ration of water, acetonitrile, and methanol, acidified with 1.5% acetic acid, at a variable flow rate of 1.3 mL/minute, with a wavelength of 368 nm was selected, obtaining a retention time of approximately 3.6 minutes, and a good chromatographic resolution (Figure 1).

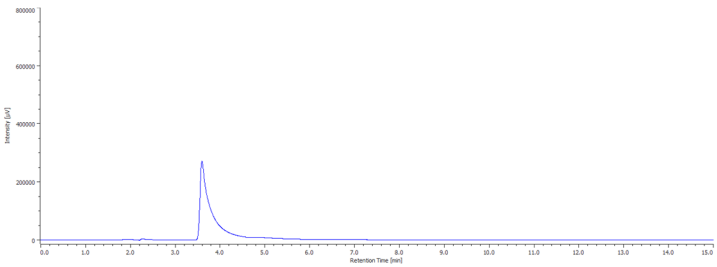

Figure 1. Chromatogram of quercetin (185 µg/mL) exhibiting the retention time at 3.6 minutes.

The method, with the defined conditions, evaluated according to the guidelines defined by the ICH, shown to be linear over a concentration range of 0.14 – 245 µg/mL, with a correlation coefficient higher than 0.995 [6]. Regarding precision (intra and inter-day), this was determined using the coefficient of variation, with the results showing values lower than 15% for all tested concentrations (0.35 µg/mL, 0.57 µg/mL, 5 µg/mL, 125 µg/mL, and 185 µg/mL), as recommended by ICH [6]. For accuracy and stability, a 15% variation is defined by the regulatory authorities as tolerable. These was also verified in all concentrations evaluated for accuracy (0.35 µg/mL, 0.49 µg/mL, 0.57 µg/mL, 49 µg/mL, 125 µg/mL, and 196 µg/mL), and stability (0.57 µg/mL, 5 µg/mL, and 125 µg/mL at – 20C, 4C, and room temperature) [6]. With the selected chromatographic conditions, the method proved to be capable of discriminating QUE from other compounds of similar chemical structure, deeming its selectivity.

4 Conclusions

Given the need to develop new formulations that allow the use of QUE in the pharmaceutical industry, the optimization of analytical techniques for its detection in various matrices becomes imperative. In this work, a HPLC-DAD method for QUE detection was successfully optimized, achieving a good correlation of characteristics that allow a correct detection, identification, and quantification of the analyte, through the use of a MP consisting of water, acetonitrile, and methanol (50:40:5), with 1.5% acetic acid, at a flow rate of 1.3 mL/minute, obtaining good results when evaluated the parameters defined by ICH.

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