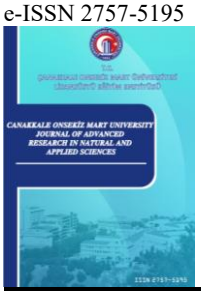
e-ISSN 2757-5195



Çanakkale Onsekiz Mart University
Journal of Advanced Research in Natural and Applied Sciences
Open Access

doi.org/10.28979/jarnas.1129991 2022, Vol. 8, Issue 4, Pages: 695-702 dergipark.org.tr/tr/pub/jarnas

Validation of Colchicine Assay Method for Therapeutic Drug Monitoring in Human Plasma

Fadime Canbolat[1,*]

[1]Department of Pharmacy Services, Vocational School of Health Services, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

Article History

Received: 13.06.2022

Accepted: 29.07.2022

Published: 15.12.2022

Research Article

Abstract - Colchicine (COL) reduces the frequency of attacks in Familial Mediterranean Fever (FMF) patients and is effective in preventing and arresting renal amyloidosis in most patients. COL has a narrow therapeutic window. The blood concentration to achieve therapeutic effects can be determined by Therapeutic Drug Monitoring (TDM). However, the use of selective and sensitive analytical methods is necessary for achieving success with TDM. The purpose of this study is to develop and validate a new method for quantitative assay of COL in human plasma samples by liquid chromatograph- tandem mass spectrometry (LC-MS/MS). In our study, to 1ml plasma sample, 0.25 ml internal standard solution was added. The solution was extracted by liquid-liquid extraction (LLE). The method was validated according to the European Medicines Agency (EMEA). The total run time was 8 min in LC-MS/MS. The method has been validated over the 0.25 - 8.0 ng/mL calibration range for COL. It was seen that the method has been validated since the results of the analysis meet the EMEA criteria. In our study, COL plasma levels were found to be approximately 1.097±0.42 ng/ml in 40 FMF patients using an oral dose of 1.5-2 mg/day. A validated, rapid, simple, cost-effective, and sensitive LC-MS/MS method was developed and optimized for quantitation of COL in plasma. It has been thought that pharmacokinetic studies of COL in plasma can be performed easily using this validated method

Keywords *– Colchicine, plasma, therapeutic drug monitoring, validation,, LC-MS/MS*

1. Introduction

The pyrin protein is responsible for producing fever symptoms in people with Familial Mediterranean Fever (FMF). Mutations in the MEFV gene can lead to this disease (Cerquaglia et al., 2005). Colchicine (COL; $C_{22}H_{25}NO_6$) reduces the frequency of attacks in FMF patients and is effective in preventing and arresting renal amyloidosis in most patients (Niel &Scherrmann, 2006; Cocco, Chu & Pandolfi, 2010). COL is a neutral, lipophilic alkaloid derived from the plant *Colchicum Autumnale*. In oral administration, it is rapidly and easily absorbed from the gastrointestinal tract. The maximum peak plasma concentration of COL, after oral administration, is reached within 0.5-2 hours (Tateishi et al., 1997). It is mainly absorbed in the ileum. Liver Cytochrome P450 (CYP) enzymes are effective in COL biotransformation (Angelidis et al., 2018). COL is metabolized to inactive metabolites which are 2-demetyl colchicine and 3-demetyl colchicine by CYP3A4 within 48 hours (Lidar et al., 2004; Wason, Di Giacinto & Davis, 2012). COL is eliminated by biliary excretion, 20 % of an oral dose is recovered unchanged in the urine. Elimination half time of COL ($T_{1/2}$) is about 23-41 hours. COL has a narrow therapeutic window because the therapeutic level of COL is 0.5-3 ng/ml and the toxic level of COL is 5 ng/ml (Niel & Scherrmann, 2006). It can lead to acute, subacute, and chronic toxicity. To achieve therapeutic effects and avoid toxic effects, drug blood concentration analysis is widely used in Therapeutic Drug Monitoring (TDM). Knowing the monitoring of the drug's blood level by physicians increases the benefits of rational drug therapy. The use of TDM has become the norm to optimize the prescribed

[1] fadime.canbolat@comu.edu.tr

[*]Corresponding Author

dose for a large number of drugs. However, the use of selective and sensitive analytical methods is necessary for achieving success with TDM. The purpose of this study is to develop and validate a new method for quantitative assay of COL in human plasma samples. In this way, a successful TDM follow-up for COL can be performed using the validated method.

2. Materials and Methods

2.1. Standards and Reagents

Colchicine (COL), and the deuterated compounds used as an internal standard colchicine-d3 (COL-d3) were purchased from Sigma (Sigma-Aldrich, St. Louis, Missouri, USA). Also, all HPLC solvents and organic solvents were purchased from Merck (Merck, Kenilworth, New Jersey, USA).

2.2. Preparation of Calibration Standards and Quality Control Samples (QCs)

The stock standard solution was prepared by dissolving COL in methanol (c: 0.1 mg/ml). The diluted standard solution (100 ng/ml) was obtained by diluting the stock standard solution of COL with methanol: water [30:70, v/v]. Calibration standards and quality control samples (QCs) were prepared using this diluted standard solution. The COL-d3 internal stock standard solution was prepared by dissolving COL-d3 (as internal standard) in methanol (c: 0.01 mg/mL). The diluted internal standard solution (c: 10 ng/mL) was obtained by diluting the internal stock standard solution of COL-d3 with methanol: water [30:70, v/v]. All solutions were stored at 4°C until required. Diluted standard solution of COL was added to different volumes of blank plasma to prepare seven calibration standards and five QCs. The calibration standards included concentrations of 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 ng/mL for COL. QCs included concentrations of 0.25, 0.75, 4.0, 6.0, 8.0 ng/mL for COL.

2.3. LC-MS/MS Conditions

HPLC was performed on an Agilent 1200 HPLC system. COL was performed using an X Terra RP18 analytical column (3mm*150mm; 3.5mm). Chromatographic separation was performed using the gradient mobile phase system. Mobil phase A was deionized water with formic acid [250:1, v/v], and mobile phase B methanol. The flow rate was 0.5 mL/min under the following conditions: 10% B held 1 min, then increased to 80 % B up to 6 min, and re-equilibrated for 2 min at initial conditions. The column was held at 38°C. MS/MS was performed on an Agilent 6410 B triple-quadrupole LC-MS/MS. Quantitative analysis was performed using multiple reaction modes with electrospray positive ionization (ES+) for COL and COL-d3. Quantification was based on monitoring the parent and product ions of COL m/z 400.2 > 310.3 and COL-d3 m/z 403.3 > 359.2, as shown in Table 1.

Table 1
Quantitation parameters for COL and COL-d3

Compound name	Retention time (RT, min)	Parent (m/z)	Quantifier product mass (m/z)	Fragment voltage (V)	Quant collision energy (eV)	Cell Accelerator voltage (V)	Polarity
COL	3.05	403.3	359.2	80	20	7	+
COL-d3	3.05	400.2	310.3	85	25		

COL; Colchicine, COL-d3; Colchicine-d3 (as internal standard)

2.4. Extraction Method of COL from Plasma Sample

To 1ml plasma sample, 0.25 ml internal standard solution (COL-d3; (10 ng/ml)) was added. The solution was extracted with 8 ml ether: dichloromethane [70:30; v/v] and mixed by vortex for 15 min. The phases were centrifuged for 1 min, 4000 rpm. The organic phase was then evaporated to dryness under a stream of nitrogen at 40°C. The residue dissolved in 250 µl methanol was injected 30 µl into the system.

2.5. Validation of COL Method

The method was validated for selectivity, calibration curve range, accuracy and precision, matrix effect, stability, and lower limit of quantification (LLOQ) according to the European Medicines Agency (EMEA) guidelines for bioanalytical methods validation and other literature that has done bioanalytical method validation studies (Jones, Singer & Bannach, 2002; Chèze, Deveaux & Pèpin,2006; EMEA, 2011; Gowda, 2014). In the selectivity study, the blank plasma sample and the lowest level of calibration standard sample were compared at COL and internal standard retention time. In the calibration curve study, seven calibration standards were analyzed by using the extraction method of COL from the plasma sample. In the accuracy and precision study, five QCs were analyzed by using the extraction method of COL from a plasma sample. The QC1 level (0.25 ng/mL) was the same as the level of the lowest calibration standard (0.25 ng/mL). The QC2 level (0.75 ng/mL) was three times the lowest calibration standard (0.25 ng/mL). The QC3 level (4 n/mL) was the middle level of the calibration curve range. The QC4 level (6 ng/mL) was about 80% of the highest calibration standard (8 ng/mL). The QC5 level (8 ng/mL) was the same as the level of the highest calibration standard (8 ng/mL). Six samples were prepared from each QC sample for intraday (within) batch analysis. 18 samples were prepared for between-batch (batch to batch) analysis. In the matrix effect study, QC2 and QC4 prepared in plasma were compared with QC2 and QC4 prepared in methanol. In the stability analysis, QC2 and QC4 samples kept at room temperature for 72 hours were compared with fresh prepared QC2 and QC4 samples. For the LLOQ analysis, it was noted that the signal-to-noise ratio was about 10 and was higher than the interference area in the blank sample.

2.6. Using The Validated Method for TDM Analysis

In our previous studies (2015a, 2015b), the plasma samples of 40 FMF patients treated at the Marmara University Training and Research Hospital Rheumatology Unit have been analyzed by this developed method (Canbolat et al., 2015a; Canbolat et al., 2015b). We have applied to the Ethics Committee of Marmara University and this study has been approved by the Ethics Committee of Marmara University. We have applied to Marmara University Ethics Committee to analyze patient blood samples. Our ethics committee application was approved by protocol no MAR-YC-2013.0179. To determine TDM, 2 mL blood samples were drawn from each patient 30 minutes before the next COL dose. All plasma samples were stored at -20° C until required.

3. Results and Discussion

3.1. Validation of COL Assay Method

Selectivity

A good analytical method should be able to separate the analytes from the endogenous components in the matrix. Selectivity should be proven by using at least six different plasma sources to find the appropriate sample of blank plasma to be used in the analysis. The criteria of the selectivity study are that the interference area in the blank sample is less than 20% of the LLOQ area and 5% of the internal standard area (EMEA, 2011). In our study, blank plasma and LLOQ samples were analyzed by using the extraction method of COL from a plasma sample. As a result of the analysis, it was determined that there was no peak in the blank sample and methanol at the retention time (RT) of approximately 3.05 minutes for both COL and internal standard as seen in Figure 1. It was seen that the method with good selectivity has been developed since the results of the analysis meet the EMEA selectivity criteria (Jones, Singer & Bannach, 2002; Chèze , Deveaux & Pèpin,2006; EMEA, 2011; Gowda, 2014).

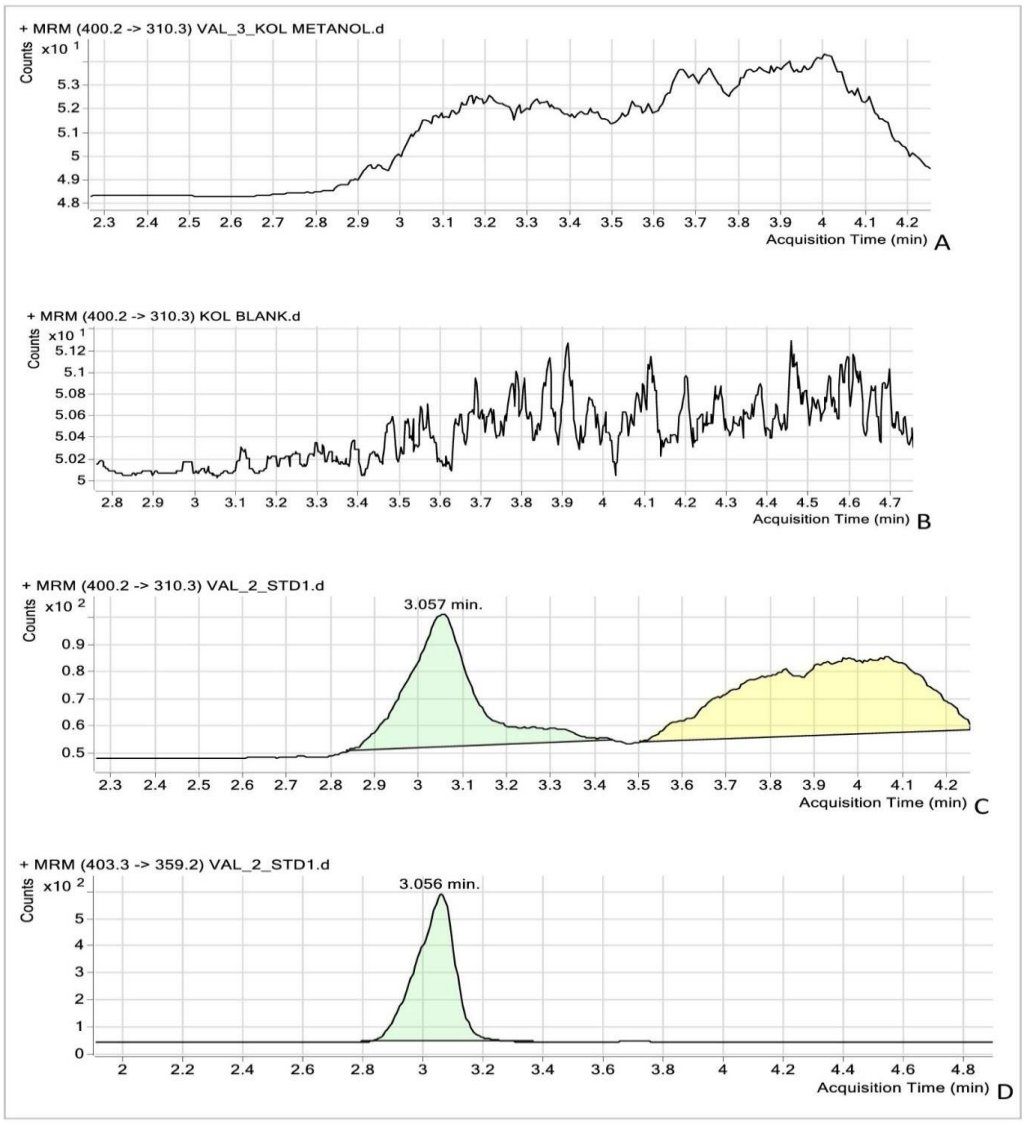


Figure 1. Chromatograms of the analysis samples. Chromatogram of methanol (as a solvent) (A); Chromatogram of blank sample (B); Chromatogram of COL (LLOQ) m/z: 400.2 > 310.3, RT: 3.05 min. (C); Chromatogram of COL-d3 m/z: 403.3 > 359.2, RT: 3.05 min. (as an internal standard) (D).

Calibration curve range

Before performing the validation of the analytical method, it should be known in which concentration range it will be studied. This calibration curve should be covered by the lowest calibration standard and the highest calibration standard. Calibration standards with at least six different concentrations should be used. Along with the standard samples, blank and zero samples should also be analyzed. According to EMEA criteria, the calculated LLOQ level must be within ± 20% of the nominal value of LLOQ. The calculation of the other calibration standard level must be within ± 15 % of the nominal value of standards. At least 75% of the calibration standards must meet this criterion.

In our study, the method has been validated over the 0.25 - 8.0 ng/mL calibration range for COL. The calibration range was established with seven calibration standards and five QCs. The calibration curve was calculated by a least-squares linear regression model. Out of seven standards, only one standard was out of the nominal value. Since the calculated standards and QC values were close to the nominal values, the calibration curve was accepted. The calibration curve are given in Figure 2. It was seen that the method with a good calibration curve has been developed since the results of the analysis meet the EMEA criteria (Jones, Singer & Bannach, 2002; Chèze , Deveaux & Pèpin,2006; EMEA, 2011; Gowda, 2014).

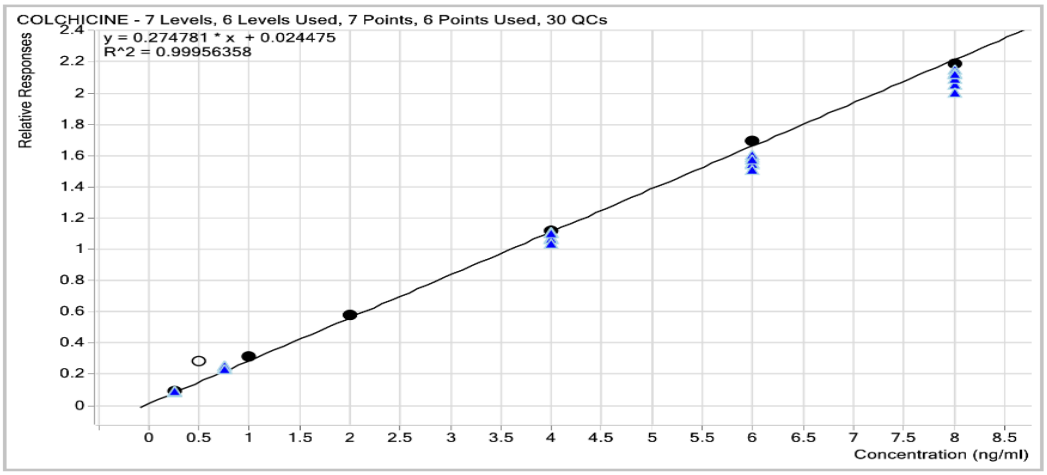


Figure 2. Calibration curve. Small circles represent the calibration standard, and small arrows represent QCs. Calibration standards and QC values were accepted when they were close to nominal values: ±15% for the high and middle levels and ±20% for both the LLOQ and lowest QC level.

Accuracy and Precision

The accuracy of an analytical method is that the calculated analyte value is close to the nominal concentration of the analyte. It should be assessed on QCs. The QCs are analyzed according to the calibration curve and the calculated QC levels are compared with the nominal value. Accuracy is assessed among QCs obtained within the batch and between batches. According to EMEA criteria, the calculated QC1 level must be within ± 20% of the nominal value of QC1. The calculation of the other QC levels must be within ± 15 % of the nominal value of QCs.

In our study, within-batch accuracy was determined by analyzing in a single run six samples per level at five QC levels. Also, between-batch accuracy was determined by analyzing in different three runs. The mean concentration was within ± 15% of the nominal values for the QCs in the intra- day (within batch) and inter-day (between batch or batch to batch). The results are given in Table 2.

The precision of the analytical method is determined by the close of the repeated analyte measurements to each other. It is the random error of accuracy (coefficient of variation; % CV). Precision is assessed among to QCs obtained within the batch and between batches. We have found that the CV value was lesser than 15% for the QCs in intra-day (within batch) and inter-day (between batch or batch to batch) (Table 2). It was seen that the method with good accuracy and precision has been developed since the results of the analysis meet the EMEA criteria (Jones, Singer & Bannach, 2002; Chèze , Deveaux & Pèpin,2006; EMEA, 2011; Gowda, 2014).

Table 2.
Intra-day and Inter-day statistics of precision and accuracy for QCs of COL

Intra-day	Correlation Coefficient (r^2)	Mean % Accuracy Range (QCs)					Precision (% CV) Range (QCs)				
		0.25 ng/mL	0.75 ng/mL	4 ng/mL	6 ng/mL	8 ng/mL	0.25 ng/mL	0,75 ng/mL	4 ng/mL	6 ng/mL	8 ng/mL
Batch 1	0.99597	95.85	100.09	104.71	104.18	104.51	5.88	6.82	1.28	3.57	2.65
Batch 2	0.99792	94.85	102.64	96.74	93.68	96.17	5.16	4.17	1.12	3.65	3.81
Batch 3	0.99956	99.49	108.14	95.82	93.35	94.05	4.49	3.98	2.56	2.16	2.54
Inter-day analysis results											
		Mean % Accuracy Range (QCs)					Precision (% CV) Range (QCs)				
Batch to batch		0.25 ng/mL	0.75 ng/mL	4 ng/mL	6 ng/mL	8 ng/mL	0.25 ng/mL	0.75 ng/mL	4 ng/mL	6 ng/mL	8 ng/mL
		96.78	103.62	99.09	97.07	98.25	5.26	5.95	4.55	5.99	5.46

Matrix Effect

Matrix effects should be determined when using mass spectrometric methods. The internal standard normalized matrix factor (IS normalized MF) is used to determine the matrix effect. The CV of IS normalized MF

calculated for the QCs should not be greater than 15%. The matrix factor was calculated according to the calculation specified in the EMEA guideline (EMEA, 2011).Firstly, the matrix factor (MF) is calculated for the analyte as seen in Equation 3.1 and IS as seen in Equation 3.2. Then, The IS normalized MF is calculated by dividing the MF of the analyte by the MF of the IS (EMEA, 2011).

$MF_{analyte}$=peak area of analyte in blank/ peak area of analyte in methanol (3.1)

MF_{IS}=peak area of IS in blank/ peak area of IS in methanol (3.2)

In our study, this calculation was done at a low and at a high level of QC. The CV of the IS-normalised MF was calculated lesser than 15 %. It was seen that the method with a good matrix effect has been developed since the results of the analysis meet the EMEA criteria (Jones, Singer & Bannach, 2002; Chèze, Deveaux & Pèpin,2006; EMEA, 2011; Gowda, 2014).

Stability

The QCs prepared stability study was analyzed against a calibration curve. Room temperature stability was performed at low QC and high QC levels, and the CV values were within ±15 %. The stability results in our study were compared with the EMEA criteria. It has been shown that the samples prepared are stable at room temperature for over 72 hours.

LLOQ

LLOQ of the method is the lowest concentration level with suitable precision and accuracy. LLOQ for COL in human plasma was set at 0.25 ng/mL. The chromatograms are given in Figure 1.

When we look at the studies conducted in the last 30 years, there are studies on the plasma level of COL. In the Tracqui et al. (1996) study, the LLOQ of COL in plasma was 0.6 ng/ml (Tracqui et al., 1996). In the Bourgogne et al. (2013) study, the run time of COL analysis was 9.5 minutes and the LLOQ level was 0.3 ng/ml (Bourgone et al., 2013). In the Fabresse et al. (2017) study, the LLOQ of COL level was determined as 0.5 ng/ml (Fabresse et al., 2017). In our study, the LLOQ of COL level was 0.25 ng/ml, and run time was 8 minutes. Considering the short run time of COL analysis and LLOQ of COL level of the validated method, a fast and sensitive analysis method has been developed compared to other studies. Even though COL has a narrow therapeutic window, analytical research on this drug is limited. The research mostly includes toxic case reports. In the Goldbart et al. study, a case report of poisoning with the use of 2 mg of COL was included. It has been shown that the cause of intoxication was the interaction of the drug with nutrients, as a result of which the level of COL was higher than expected (Goldbart, Press, Sofer &Kapelushnik, 2000). In our previous studies, COL plasma levels were found to be approximately 1.097±0.42ng/ml in 40 FMF patients using an oral dose of 1.5-2 mg/day (Canbolat et al., 2015a; Canbolat et al., 2015b). Since the therapeutic plasma concentration of COL is relatively low (0.5-3 ng/ml), many analytical studies (non-specific radioimmunoassay, chromatography methods) for the determination of COL to date have lagged behind expectations for measurements at the therapeutic level. Physicians need to determine the levels of COL in the patient's plasma to ensure an appropriate and safe therapeutic dose. The fact that the plasma level of COL can be analyzed with a rapid and sensitive method becomes important to achieving the therapeutic effect of COL. Considering the superiority of the validated method over other COL analysis methods in the literature, it is thought that the validated method can be easily used in TDM analysis.

4. Conclusion

Current studies show the importance of plasma drug monitoring both to evaluate the clinical response and to prevent drug toxicity. We, therefore, decided to develop a highly specific and sensitive LC-MS/MS method for the determination and quantitation of COL in plasma. A validated, rapid, simple, cost-effective, and accurate LC-MS/MS method was developed and optimized for the quantitation of COL in plasma. It is also a practical and easy method for pharmacokinetic studies involving the evaluation of TDM of COL.

Acknowledgment

I would like to thank Gulsen Ozen, Ali Ugur Unal, Guzide Nevsun Inanc, Pamir Atagunduz, and Haner Direskeneli for supporting the collection of patient blood samples in our previous study. Thank you Selma Ozilhan, Kasif Nevzat Tarhan, and deceased Salih Tuncel Ozden for providing laboratory facilities.

Author Contributions

Fadime Canbolat: The author takes part in the development and validation of the colchicine assay method, analysis of samples, and writing of the manuscript.

Conflicts of Interest

The author declares no conflict of interest.

References

Angelidis, C., Kotsialou, Z., Kossyvakis, C., Vrettou, A. R., Zacharoulis, A., Kolokathis, F., Vasilios, K., & Giannopoulos, G. (2018). Colchicine pharmacokinetics and mechanism of action. *Current pharmaceutical design*, *24*(6), 659-663. doi: https://doi.org/10.2174/1381612824666180123110042

Bourgogne, E., Soichot, M., Latour, C., & Laprévote, O. (2013). Rugged and accurate quantitation of colchicine in human plasma to support colchicine poisoning monitoring by using turbulent-flow LC–MS/MS analysis. *Bioanalysis*, *5*(23), 2889-2896. doi: https://doi.org/10.4155/bio.13.258

Canbolat, F., Ozen, G., Ozilhan, S., Gulturk, S., Ozcetin, A., Unal A.U., Inanc, N., Atagunduz, P., Direskeneli, H., & Ozden, T. (2015a). Relationship Between Colchine Plasma Level and Frequency of Familial Mediterranean Fever Attacks. *ACR/ARHP Annual Meeting*, Abstract 941, USA. Retrived from: https://acrabstracts.org/abstract/relationship-between-colchine-plasma-level-and-frequency-of-familial-mediterranean-fever-attacks/

Canbolat, F., Ozen, G., Ozilhan, S., Gulturk, S., Ozcetin, A., Unal, A. U., Inanc, N., Atagunduz, P., Direskeneli, H., & Ozden, T. (2015b). THU0549 Relationship Between Colchine Plasma Level and Frequency of Familial Mediterranean Fever Attacks.BMJ Journals Annals of The Rheumatic Diseases, 74 (2). Retrived from:https://ard.bmj.com/content/74/Suppl_2/399.2

Cerquaglia, C., Diaco, M., Nucera, G., Regina, M. L., Montalto, M., & Manna, R. (2005). Pharmacological and clinical basis of treatment of Familial Mediterranean Fever (FMF) with colchicine or analogues: an update. *Current Drug Targets-Inflammation & Allergy*, *4*(1), 117-124. doi: https://doi.org/10.2174/1568010053622984

Cocco, G., Chu, D. C., & Pandolfi, S. (2010). Colchicine in clinical medicine. A guide for internists. *European journal of internal medicine*, *21*(6), 503-508. doi: https://doi.org/10.1016/j.ejim.2010.09.010

Chèze, M., Deveaux, M., & Pèpin, G. (2006). Liquid chromatography-tandem mass spectrometry for the determination of colchicine in postmortem body fluids. Case report of two fatalities and review of the literature. *Journal of analytical toxicology*, *30*(8), 593-598. doi: https://doi.org/10.1093/jat/30.8.593

EMEA Committee for Medicinal Products for Human Use (2011). *Guideline on bioanalytical method validation. European Medicines Agency EMEA*. CHMP/EWP/192217/2009: 1-22 (cited 2011 August 01). Retrived from: www. ema. europa. eu/ema/pages/includes/document/open_document. jsp.

Fabresse, N., Allard, J., Sardaby, M., Thompson, A., Clutton, R. E., Eddleston, M., & Alvarez, J. C. (2017). LC–MS/MS quantification of free and Fab-bound colchicine in plasma, urine and organs following colchicine administration and colchicine-specific Fab fragments treatment in Göttingen minipigs. *Journal of Chromatography B*, *1060*, 400-406. doi:https://doi.org/10.1016/j.jchromb.2017.06.034

Goldbart, A., Press, J., Sofer, S., & Kapelushnik, J. (2000). Near fatal acute colchicine intoxication in a child. A case report. *European journal of pediatrics*, *159*(12), 895-897. doi:https://doi.org/10.1007/PL00008364

Gowda, B. G. (2014). High-Performance liquid chromatographic determination of colchicine in pharmaceutical formulations and biological fluids. *Int. J Pharm Pharm Sci*, *6*, 335-337. Retrived from:https://web.archive.org/web/20180413121507id_/http://ijppsjournal.com/Vol6Issue6/9624.pdf

Jones, G. R., Singer, P. P., & Bannach, B. (2002). Application of LC-MS analysis to a colchicine

fatality. *Journal of analytical toxicology*, *26*(6), 365-369. doi: https://doi.org/10.1093/jat/26.6.365

Lidar, M., Scherrmann, J. M., Shinar, Y., Chetrit, A., Niel, E., Gershoni-Baruch, R., Langevitz, P., & Livneh, A. (2004). Colchicine nonresponsiveness in familial Mediterranean fever: clinical, genetic, pharmacokinetic, and socioeconomic characterization. In *Seminars in arthritis and rheumatism* (Vol. 33, No. 4, pp. 273-282). WB Saunders. doi: https://doi.org/10.1053/S0049-0172(03)00137-9

Niel, E., & Scherrmann, J. M. (2006). Colchicine today. *Joint Bone Spine*, *73*(6), 672-678. doi: https://doi.org/10.1016/j.jbspin.2006.03.006

Tateishi, T., Soucek, P., Caraco, Y., Guengerich, F. P., & Wood, A. J. (1997). Colchicine biotransformation by human liver microsomes: identification of CYP3A4 as the major isoform responsible for colchicine demethylation. *Biochemical pharmacology*, *53*(1), 111-116. doi:https://doi.org/10.1016/S0006-2952(96)00693-4

Tracqui, A., Kintz, P., Ludes, B., Rouge, C., Douibi, H., & Mangin, P. (1996). High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids. *Journal of Chromatography B: Biomedical Sciences and Applications*, *675*(2), 235-242. doi: https://doi.org/10.1016/0378-4347(95)00386-X

Wason, S., DiGiacinto, J. L., & Davis, M. W. (2012). Effects of grapefruit and Seville orange juices on the pharmacokinetic properties of colchicine in healthy subjects. *Clinical therapeutics*, *34*(10), 2161-2173. doi: https://doi.org/10.1016/j.clinthera.2012.08.007