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Original Article

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF MEFENAMIC ACID IN HUMAN PLASMA USING UV VIS DETECTOR

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ABSTRACT

Objective: Mefenamic Acid (MA) is a non-steroidal anti-inflammatory drug (NSAIDs). This drug provides analgesic and antipyretic (fever reducing effect) and higher doses, anti-inflammatory effect. This study is focused to develop a rapid and sensitive method for the detection of mefenamic acid in human plasma.

Methods: Protein precipitation technique using acetonitrile was used. Chromatographic separation was achieved on Agilent Zorbax Eclipse XDB-C_{18} (150 mm x 4.6 mm, i. d 3.5 µm) with a mobile phase consisting of acetonitrile and 2% triethylamine (pH was adjusted to 4.2 with phosphoric acid) in a ratio of 60:40. The retention time for mefenamic acid and diclofenac was 5.4 and 3.9 minutes respectively. The mefenamic acid was monitored at 280 nm using variable-wavelength detector.

Results: The recovery was found 83% for MA. The method was validated according to the Centre for Drug Evaluation and Research (CDER) guidelines. Calibration plot was linear within the range from 250 to 5000ng ml^{-1} with the coefficient of determination (r^2) of ≥ 0.99. The quality control samples of mefenamic acid which was termed as low (L), medium (M) and high (H) were analysed to get the precision and accuracy. The accuracy for intra-day for L, M and H was 99.71%, 93.8% and 89.52% while for inter day were 97.67%, 93.46% and 91.67% respectively. On the other hand, coefficient variance (CV) for intra-day precision for L, M and H was found 2.57%, 2.45% and 1.45% and for inter day CV were 3.11%, 5.5% and 4.37% respectively. Diclofenac sodium was used as internal standard for this study.

Conclusion: The results were in compliance with CDER guideline.

Keywords: HPLC, Mefenamic Acid, Human plasma, UV detector, Protein precipitation.

INTRODUCTION

Mefenamic acid (benzoic acid, 2-(2, 3-dimethylphenyl) amino-N-2, 3-Xylylanthranilic acid) is non-steroidal anti-inflammatory drugs (NSAIDs)[1]. Mefenamic acid acts on cyclooxygenase-2 and inhibits prostaglandin synthesis. It is used in the treatment of headache, dental pain, postoperative and postpartum pain, and many more.

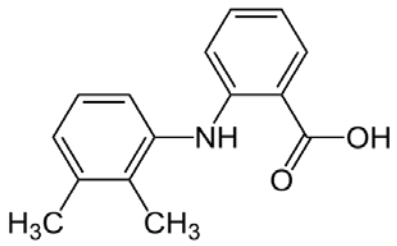

Fig. 1: Chemical structure of mefenamic acid

The usual dose of mefenamic acid is 250 – 500 mg given three times a day. It is rapidly absorbed after oral administration. C_{max} of mefenamic acid is 20 µg ml^{-1} and this state can last for 2-4 hour. There is several reported study of using HPLC and GC for detecting mefenamic acid in human serum and urine. For protein precipitation technique [2, 3], according to these methods, retention time for MA was longer which was more than 10 minutes. Liquid - liquid extraction technique [4-6] required large sample volume, and are not very suitable for routine bioanalysis. Besides that, one reported study involves multiple extraction which is time consuming. In other studies detection of MA in urine [7, 8] involved SPE as extracting method. SPE is costly and required large amount of sample.

This study was aimed to develop a simple, sensitive, reliable and cost effective method for detection and quantitation of mefenamic acid in human plasma.

MATERIALS AND METHODS

Chemicals and reagent

HPLC grade acetonitrile and triethylamine were obtained from Fisher scientific, phosphoric acid analytical grade was obtained from BDH Darmstadt, Germany. Ultrapure water was taken from Elga Purelab Ultra water system. Standard reference material such as mefenamic acid, diclofenac sodium, flufenamic acid and indomethacin were purchased from Sigma-Aldrich.

Apparatus

Agilent 1100 series HPLC system was used for this study which consists of degasser, binary pump, auto sampler with Rheodyne Technology, column compartment and UV/Vis detector. The whole HPLC system was controlled by Agilent Chemstation for LC 3D Rev. A. 10.02(1757)

Chromatographic condition

The chromatographic separation was performed using Agilent Zorbax Eclipse C18 column (150 mm x 4.6 mm, 3.5 µm) with C_{18} analytical guard column. The mobile phase consists of acetonitrile and 2 % triethylamine adjusted to pH 4.2 with phosphoric acid (85%) with ratio of 60:40 (v/v) and was delivered at a flow rate 1 ml min^{-1}. The solution was filtered using 0.45 µm nylon membrane prior to use. CDER guideline was adopted for chromatographic method validation [9].

Standard solution

Stock solution of 0.1 mg ml^{-1} mefenamic acid and diclofenac sodium (internal standard) was dissolved in acetonitrile. The stock solution

was diluted to obtain 0.01 mg ml^{-1} of MA and diclofenac sodium and used as working standard solution.

Extraction procedure

500 µl of spiked standard plasma with mefenamic acid was taken into 5 ml glass tube, 4000 ng ml^{-1}diclofenac sodium (IS) was added to the spiked plasma. 2 ml of acetonitrile was added to the mixture. The samples were vortexed for 30 sec and centrifuged for 5 minutes at 5000 rpm. The clear supernatant was transferred to another tube and evaporated to dryness at 40^{0}C under stream of nitrogen. The residue was reconstituted with 200 µl of mobile phase and used for analysis.

Mobile phase composition

The mobile phase composition was optimized using acetonitrile and combination of several types of buffer in different composition and pH adaption in isocratic mode for the mefenamic acid. The combination of mobile phase was selected based on the retention time factor as well as capability of detection for lower detection.

Internal standard

Several compounds were targeted to obtain a clear baseline to serve as an internal standard (IS). The compounds were flufenamic acid, diclofenac sodium and indomethacin. The compound that showed good recovery, better compatibility with the extraction method, and shorter analysis time was selected as an internal standard for mefenamic acid.

Selectivity and specificity

The lack of chromatographic interference at retention times of mefenamic acid and IS from endogenous plasma components was investigated. Six different batch of human plasma were analysed for selectivity and specificity following the optimized chromatographic method.

Calibration

The spiked plasma containing standard mefenamic acid 250, 500, 1500, 2500, 3500 and 5000 ng ml^{-1} was prepared for linearity. Calibration curve was prepared by plotting the peak area ratio of mefenamic acid and internal standard against the mefenamic acid concentration (ng ml^{-1}). The coefficient of determination (r^2) must be 0.99.

Limit of detection and limit of quantitation

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value. A signal to noise ratio of 3:1 is considered acceptable for estimating LOD. The limit of quantification (LOQ) was the lowest point of the calibration curve which could be detected and its best signal to noise ratio is 10:1.

Precision and accuracy

Method performance needs to be evaluated using precision and accuracy. Precision is defined by the measure of the coefficient of variation of the QC samples. The accuracy is defined as the trueness of the value of QC sample to the true value. Analysing 6 replicate at four different QC levels (LLOQ, L, M, H which was 250 ng, 400 ng, 2700 ng, 4500 ng respectively) were analysed for intraday precision and accuracy. The inter-day precision and accuracy were analysed with three replicates at four QC level concentrations on 8 different days.

Recovery

The recovery of protein precipitation technique was calculated via comparing the response area of the mefenamic acid in spiked plasma and its corresponding standard solution and multiply by 100.

Recovery (%) = [area response of MA in spiked plasma/area response of corresponding MA standard] X 100.

Stability

The samples were stored at -40^{0}C freezer. The stability of each sample was compared with the fresh sample.

Freeze and thaw stability

The QC samples were taken from -40^{o}C freezer and thawed unassisted at 25^{o}C room temperature. After thawing, the samples were re frozen for 12-24 hours. This cycle was repeated three times. QC sample (LLOQ, Low, Medium and High) was analyzed until the third cycle.

Short-term temperature stability

The QC samples were thawed at room temperature (25^{o}C) for 10 hours and analyzed.

Long term stability

The QC samples were stored at -40^{o}C for 1, 2 and 3 months and analyzed.

RESULTS AND DISCUSSION

Specificity and selectivity

We demonstrated the absence of interfering endogenous compound in blank plasma. Figure 2a showed blank plasma chromatogram. Addition of IS in figure 2b showed that they were well separated with no interfering peaks. Addition of mefenamic acid with IS (figure 2c) showed that mefenamic acid is well resolved with IS and no interference occurred.

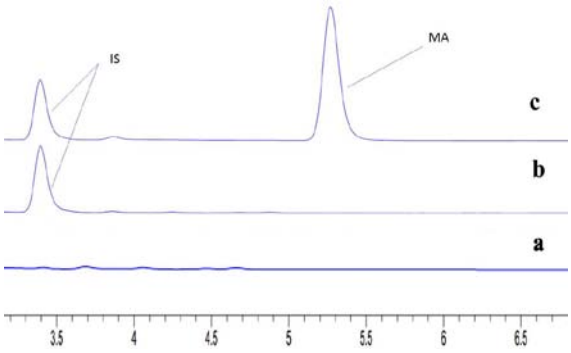

Fig. 2: Chromatograms obtained from (a) blank plasma (b) plasma spiked with 4000 ng ml^{-1} diclofenac sodium (IS) (c) plasma spiked with IS and MA, respectively

Linearity

Mefenamic acid was spiked in plasma to obtain the calibration standard. 6 calibration points ranging from 250 – 5000ng ml^{-1} were prepared. All calibration point contained 4000ng ml^{-1} of diclofenac sodium (internal standard). The calibration curve was prepared by ratio of mefenamic acid and IS (peak area of analyte of mefenamic acid/peak area of internal standard). The standard curve was linear over the range 250 – 5000 ng ml^{-1}. The standard curve was calculated by linear regression method: y = ax + b where y is the peak area ratio of drug to an internal standard, a and b are constant, and x is the mefenamic acid concentration(ng ml^{-1}).

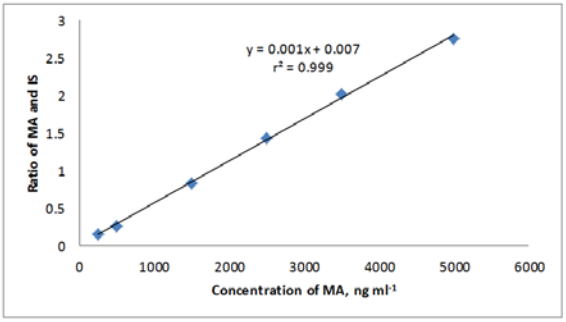

Fig. 3: Typical of calibration curve of MA in plasma

Limits of detection

The minimum detectable concentration of mefenamic acid was found to be 70 ng ml^{-1} whereas the quantitative limit (LOQ) was 250ng ml^{-1}

Accuracy and precision

Method performance of accuracy and precision was evaluated as intraday and interday accuracy precision. It was determined by replicate analysis of QC samples. The results obtained have been listed in Table 1 and 2. These results show good repeatability of the method used including both sample processing and chromatographic measurement. For intraday, it can be seen that the accuracy was perfect and the values were 93.8% - 110.84% whereas coefficient of variation (CV) were within 1.45 – 2.57. For interday accuracy, it was between 91.67% – 102.69% and for precision which is expressed as CV was within 3.11 % - 5.5 %.

Recovery

Recovery *was showed in table 3*. The result of the recovery is the average of 8 replicates for each concentration. The recovery for all the concentration is more than 80 %.

Table 1: Intraday (n=8) and interday (n=6) accuracy

Concentration (ng ml^{-1})	Intra day (n=8)	Inter day (n=6)
	Accuracy, %	Accuracy, %
(LLOQ) 250	110.84	102.69
(Low) 400	99.71	97.67
(Medium) 2700	93.80	93.46
(High) 4500	89.52	91.67

Relative accuracy {(amount of sample/actual value)*100}

Table 2: Intraday (n=8) and interday (n=6) precision

Concentration (ng ml^{-1})	Precision					
	Intra day (n = 8)			Inter day (n = 6)		
	Average	SD	CV	Average	SD	CV
(LLOQ) 250	277.11	7.03	2.54	256.73	12.52	4.88
(Low) 400	398.85	10.25	2.57	390.66	97.67	3.11
(Medium) 2700	2532.65	61.93	2.45	2523.35	138.67	5.5
(High) 4500	4028.39	58.57	1.45	4125.02	180.12	4.37

CV {(SD/average)*100}

Table 3: recovery (%) for every QC concentration (n=8)

Concentration (ng ml^{-1})	Recovery (%) Average
250	90.18
400	82.24
2700	80.08
4500	81.91

Table 4: Results of different types of stability samples of MA in plasma.

Types of stability	Percentage of MA concentration %.			
	LLOQ	Low	Medium	High
1. Short term stability	102.77	96.40	105.53	87.87
2. Freeze-thaw stability				
a. 1st cycle stability	92.97	92.07	99.21	94.10
b. 2nd cycle stability	89.97	90.63	98.70	95.34
c. 3rd cycle stability	91.93	91.67	101.25	94.14
3. long term stability				
a. 1st month	104.13	103.85	100.46	95.45
b. 2nd month	87.04	94.72	94.43	91.13
c. 3rd month	90.50	95.20	90.10	93.35

Stability

In the present study, the stability of short-term temperature, freeze thaw cycle and long term was demonstrated. The degradation of mefenamic acid after third freeze thaw cycle was 8%, and for short term stability was 1.91% and long term stability after 3 months was 7.7 %.

CONCLUSION

In conclusion, we have developed and validated a simple, sensitive, specific and reproducible method for mefenamic acid determination in human plasma.

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Sweetman SC. Martindale the Complete Drug Reference. London: Pharmaceutical Press; 2002.

2. Sato J, Amizuka T, Niida Y, Umetsu M, Ito K. Simple, rapid and sensitive method for the determination of indomethacin in plasma by high-performance liquid chromatography with ultraviolet detection. J chromatogr B 1997;692(1):241–4.
3. Niopas I, Mamzoridi K. Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography. J Chromatogr B 1994;656:447–50.
4. Dahivelkar PP, Bari SB, Bhoir S, Bhagwat AM. High performance liquid chromatographic estimation of drotaverine and mefenamic acid in human plasma. Iran J Pharm Res 2009;8(3):209-15.
5. Yamashita K, Motohashi M, Yashiki T. Column-switching techniques for high-performance liquid chromatography of ibuprofen and mefenamic acid in human serum with short wavelength ultraviolet detection. J Chromatogr 1991;570:329-38.
6. Sun Y, Takaba K, Kido H, Nakashima MN, Nakashima K. Simultaneous determination of arylpropionic acidic non-steroidal anti-inflammatory drugs in pharmaceutical formulations and human plasma by HPLC with UV detection. J Pharm Biomed Anal 2003;30:1611–9.
7. Hirai T, Matsumoto S, Kishi I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high performance liquid chromatography with normal solid phase extraction. J Chromatogr B 1997;692:375–88.
8. Mikami E, Goto T, Ohno T, Matsumoto H, Inagaki K, Ishihara H, *et al*. Simultaneous analysis of anthranilic acid derivatives in pharmaceuticals and human urine by high performance liquid chromatography with isocratic elution. J Chromatogr B 2000;744:81–9.
9. Center for Drug Evaluation and Research (CDER). Guidance for Industry Bioanalytical method validation. U. S Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research; 2001.