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SPECTROPHOTOMETRIC DETERMINATION OF MEFENAMIC ACID EXCRETED AS FREE DRUG IN URINE OF HUMAN BEINGS

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Abstract
Urinary excretion of free mefenamic acid was investigated in 16 healthy human volunteers, eight males and eight females, following the oral administration of 500mg tablet of mefenamic acid. Urine samples were collected at pre-determined schedule and drug concentration was determined by spectrophotometric method. The total recovery of free mefenamic acid was 1.526 ± 0.128 and 1.193 ± 0.112% in male and female volunteers respectively. The average ± S.E values for diuresis, pH and rate of excretion of mefenamic acid was 0.0160 ± 0.004mL/min./kg of body weight, 6.22 ± 0.167, 0.077 ± 0.016 μg min^{-1} kg^{-1} in male while 0.0084 ± 0.0023mL min^{-1} kg^{-1} of body weight, 6.35 ± 0.164, 0.054 ± 0.008 μg min^{-1} kg^{-1} respectively in female volunteers. The results obtained are different from the earlier studies due to variability in dose, gender variation, fluctuation in urine pH, environmental conditions and nutritional ingredients.

Keywords: Human beings, mefenamic acid, spectrophotometry, urinary excretion.

INTRODUCTION
Mefenamic acid, N-(2,3-xylyl) anthranilic acid is nonsteroidal, anti-inflammatory drug (NSAID) which has analgesic, anti-inflammatory and antipyretic actions. Mefenamic acid is used to relieve pain arising from soft–tissue injuries, menorrhagia and other painful musculoskeletal conditions [Budoff 1979, Hart and Huskisson 1984, Hall *et al.* 1987]. It is also indicated for the treatment of rheumatoid arthritis [Martindale 1982], primary dysmenorrheal [Zhang and Li 1998] and periodontitis [Cory and Moran].
Mefenamic acid is readily absorbed from gastrointestinal tract and extensively bound to plasma protein and excreted mainly in urine as conjugated metabolites. Small amount may be excreted as unchanged drug. Mefenamic acid and its metabolic derivatives become conjugated with glucuronic acid through an ester linkage, which is alkali labile, and are mainly excreted in urine but also to some extent in bile and faeces [www.medsafe.govt.nz]. A small portion (1-15%) is eliminated unchanged by the kidneys [Isselbacker *et al.* 1994]. The elimination half life of mefenamic acid is approximately two hours, half life of metabolite-1

and metabolite-II have not been precisely reported, but appear to be longer than the parent compound. The metabolites may accumulate in patients with renal and hepatic failure. The mefenamic acid glucuronide may bind irreversibly to plasma proteins, because both renal and hepatic excretions are significant pathways of elimination [www.rx.list.com]. In preterm infants undergoing mefenamic acid therapy, the cumulative amounts of mefenamic acid and the metabolites excreted in the urine varied from 7 to 46% of the total dose administered and it is found to be less than those reported in adults and children [Sato *et al.* 1997].

Titrometric method for the assay of the pure drug has been applied using sodium hydroxide as titrant and phenol red as indicator [Rawasheh *et al.* 1997, British Pharmacopoeia 1998 Vol. 1, 2]. A variety of other methods have also been used for the determination of the drug in biological fluids. Determination in the serum has been carried out with high performance liquid chromatography (HPLC) with UV detection and spectrofluorimetry [Ionnaou *et al.* 1998]. Determination in the blood has been carried out with HPLC with photodiode array detector and gas chromatography (GC) with flame ionization detector [Lo *et al.* 1997]. Determination in urine was reportedly done by HPLC [Hirai *et al.* 2002].

Trends in pharmaceutical analysis and compendial assay methods [Hirai *et al.* 2002] reflect increasing replacement of time-honoured classical methods of analysis with modern, more sophisticated instrumental methods, especially HPLC. However, the high acquisition and maintenance cost of this equipment makes adoption of assay methods based on the rather inconvenient, especially in poor-resource economies. This often warrants the use of an alternative method.

Spectrophotometry as an analytical technique with the advent of scanning spectrophotometers is very useful. By means of the monochromator, discrete wavelength interval in the ultraviolet, visible and near infra-red region can be isolated easily on spectrophotometers. These attractive features led to a resurgence of interest in colorimetric assay of pharmaceuticals. Spectrophotometry provides a unique combination of high technology and low cost in analytical methodology; it shares the typical merits of instrumental methods, especially, faster handling of large sample [Willard *et al*. 1988].

In this paper, spectrophotometric assay of mefenamic acid in the urine of human beings has been reported after oral administration of mefenamic acid tablets. Investigations have shown that biodisposition kinetics, renal clearance and urinary excretion of many drugs were different under indigenous conditions, as compared to literature values. Environmental influences on genetics, manifested through biochemical and physiological parameters, affecting fate of drugs in population are explained by term geonetics [Zhang and Li Wan 1998]. The study was planned to evaluate the urinary excretion of mefenamic acid in human beings under local conditions to measure the effects of geonetics.

MATERIALS AND METHODS

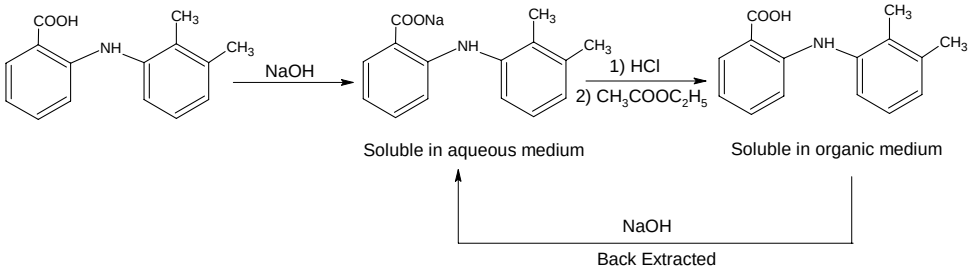
EQUIPMENT

All measurements were carried out using a Hitachi U1100 spectrophotometer (Japan) with a silica glass cell of 1 cm thickness. Officially calibrated Pyrex glassware was used throughout this study.

CHEMICALS AND REAGENTS

All chemicals and reagents used were of analytical reagent grade. Mefenamic acid was supplied by Werrick Pharmaceuticals (Pvt.) Ltd., Islamabad, Pakistan. Mefenamic acid tablets were purchased from the local market of Faisalabad, Pakistan. Water used was double distilled.

Mefenamic acid 1000 μg ML^{-1} (stock solution-1)) was prepared by dissolving 100mg of mefenamic acid in small amount of 0.1N NaOH and then making its volume up to 100 mL with distilled water. Stock solution-2 of 100 μg mL^{-1} was prepared by taking 10 mL from stock solution-1 and making its volume up to 100 mL with distilled water. Standard solutions having concentrations of mefenamic acid, 1, 2, 3, 4, 5 μg mL^{-1} were prepared by taking 0.1, 0.2, 0.3, 0.4, and 0.5 mL respectively of stock solution-2 and making their volume up to 10 mL with urine. Triplicate set was prepared for each concentration.



Scheme 1: Showing the proposed method.

PROPOSED METHOD

In 1mL of each of standard solution, 0.5 mL of 0.1N HCl was added and shaked for 30 seconds. For blank, in 1mL urine, 0.5 mL of 0.1N HCl was added and shaked for 30 seconds. Then in both, the standard solutions and blank, added 6 mL of ethyl acetate and shaked for 2 minutes for the extraction of drug into organic layer. Ethyl acetate layer was separated and 4mL of 0.1N NaOH was added to this separated organic layer for back extraction of drug. Then after shaking for 1 minute, aqueous layer was separated. Absorbance of each of the standard against blank was measured with spectrophotometer at 285 nm. Absorbance versus concentration of standards given in Table 1 was plotted and a linear curve was obtained which is shown in Fig. 1. A standard factor was also calculated as follows:

Standard factor = (Standard concentration) / Absorbance

Table 1: Absorbance of standard concentrations of mefenamic acid in urine

Concentration (μg mL^{-1})	Absorbance	Average Absorbance	Standard factor
1.	0.371, 0.373, 0.369	0.371	2.69
2.	0.733, 0.732, 0.734	0.733	2.73
3.	1.098, 1.094, 1.102	1.098	2.73
4.	1.445, 1.444, 1.446	1.445	2.77
5.	1.831, 1.836, 1.826	1.831	2.73
Average	-	-	2.73

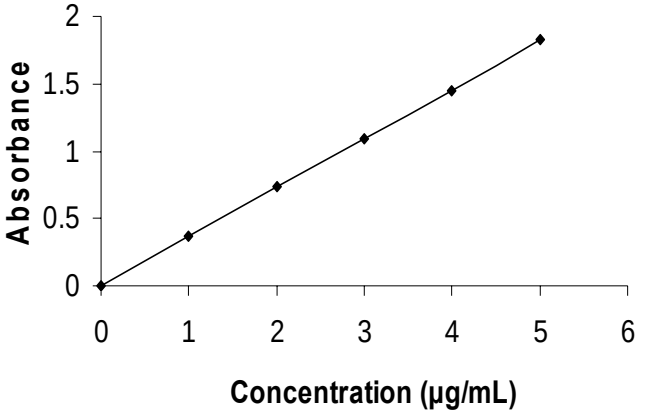


Fig. 1: Calibration curve for mefenamic acid analysis in urine.

ANALYSIS OF MEFENAMIC ACID IN URINE

The research was conducted on eight healthy male and eight healthy female volunteers. Physical parameters like age, body weight, and height of each volunteer was recorded before the start of experiment, which is given in Table 2. Each volunteer was given orally a tablet of mefenamic acid (500 mg) manufactured by Parke-Davis (Pvt.) Ltd. Co. Urine samples were collected at 0, 60, 120, 180, 240, 360, 480, 600, 720 and 1440 minutes time after drug administration. Total volume of urine and pH was noted. A small volume of each urine sample was stored in plastic bottles and preserved in freezer.

Table 2: Description of physical parameters; age, body weight and height of each volunteer.

Sr. No.	Male			Female		
	Age (Years)	Body weight (Kg)	Height (Feet)	Age (Years)	Body weight (Kg)	Height (Feet)
1	21	70	5.9	22	52	5.3
2	22	73	5.8	22	52	5.1
3	21	74	5.8	20	48	5.1
4	22	69	5.7	21	57	5.4
5	23	68	5.7	21	55	5.1
6	21	69	5.9	21	51	5.3
7	20	71	5.9	20	45	5.3
8	22	73	5.9	23	58	5.4

Urine samples were diluted ten times with distilled water and to 1 mL of diluted urine samples, 0.5 mL of 0.1N HCl was added and shaked for 30 seconds, then added 6 mL of ethyl acetate to above acidified samples and shaked for 2 minutes for the extraction of drug into organic layer. Ethyl acetate layer was separated and added to it, 4mL of 0.1N NaOH for back extraction of mefenamic acid. Then after shaking for 1 minute, aqueous layer was separated. Blank was treated in a similar manner by taking zero time urine samples. Absorbance of aqueous layers obtained, was measured by using spectrophotometer at 285 nm [Clarke 1974].

The diuresis, concentration of mefenamic acid in urine, rate of excretion, amount of mefenamic acid excreted, %age dose excreted and %age cumulative dose excreted were calculated as follows:

i) The rate of urine flow in a time period (Diuresis) was calculated as:

$$\text{Diuresis (mL min}^{-1}\text{kg}^{-1}) = \frac{\text{Volume of urine in collection period}}{\text{Time (min.) x body weight (kg)}}$$

ii) Concentration of mefenamic acid in urine was calculated by regression line:

$$Y = a + bX$$

where Y=Absorbance and X=Concentration of mefenamic acid (μg mL^{-1}).

iii) Rate of excretion (μg min^{-1}kg^{-1}) = Diuresis x (conc. of mefenamic acid excreted)

iv) Amount of mefenamic acid excreted = Uc x Uv
where Uc = Concentration of mefenamic acid in urine and Uv = Volume of urine voided.

v) $$\%\text{age dose excreted} = \frac{\text{Amount of drug excreted}}{\text{Dose of Drug (Given)}}$$

vi) $$\%\text{age cumulative dose excreted} = \frac{\text{Cumulative amount of drug excreted}}{\text{Dose of Drug (Given)}}$$

The results are given as average ± S.E [Steel and Torrie 1992].

RESULTS AND DISCUSSION

The average ± S.E. values for diuresis were 0.0160 ± 0.004 mL min^{-1}kg^{-1} of body weight and 0.0084 ± 0.0023 mL min^{-1}kg^{-1} of body weight in male and female volunteers, respectively. Both the values are different because of gender difference and volume uptake of each volunteer during sampling. The urinary system provides a most important route by which a body can excrete substances absorbed in excess of current requirements and the substances no longer required by the body, some of which are non-volatile products of tissue metabolism [Brander and Pugh 1977]. The main responsibility for adjusting solute and water excretion is borne by kidney [Tadlock 1993]. It is the major channel of water excretion as compared to intestine, skin and lungs. In kidney, a fluid that resembles plasma is filtered through glomerular capillaries into the renal tubules [Ganong 1997]. As the Glomerular filterate flows through the tubules over 99% of its water and varying amount of its solutes are normally reabsorbed into the vascular system and small amounts of some substances are secreted into the tubules, the remaining tubular water and dissolved substances become urine [Guyton and John 1996]. Tubular molar clearance, plasma osmotic pressure, antidiuretic hormone and blood pressure affect the rate of urine [Frandson 1974].

The average urinary pH ± S.E. values were 6.22 ± 0.167 and 6.35 ± 0.164 in male and female volunteers, respectively. Reaction of the main body fluid depends on the state of biochemical interior of an organism and is reflected by pH value [Cloes 1967]. Alteration of pH results in significant change in drug elimination. Weak acids are excreted more rapidly in alkaline medium, primarily because they are more ionized and passive reabsorption is decreased and vice versa [Leslie *et al*. 1996].

The concentration of mefenamic acid excreted, rate of excretion, % dose and cumulative % dose excreted in both male and female volunteers was calculated by the above given formulas.

The concentration of mefenamic acid was 7.61 ± 2.14 μg mL^{-1} and 10.93 ± 2.88 μg mL^{-1} in male and female volunteers respectively. The difference in values is due to difference in environmental temperature and urine volume. The rate of excretion of mefenamic acid was 0.077 ± 0.016 μg $min^{-1}kg^{-1}$ in male while 0.054 ± 0.008 μg $min^{-1}kg^{-1}$ respectively in female volunteers. The average percent dose of mefenamic acid excreted as free drug in eight healthy male and female volunteers given in Table 3, was 0.218 ± 0.039% in males, while in female volunteers it was 0.149 ± 0.023%.

Table 3: Percent of mefenamic acid excreted in the urine of male and female volunteers.

Time (min)	Male	Female
	Average ± SE	Average ± SE
60	0.083 ± 0.015	0.051 ± 0.008
120	0.194 ± 0.043	0.092 ± 0.009
180	0.238 ± 0.026	0.111 ± 0.012
240	0.238 ± 0.026	0.227 ± 0.047
360	0.343 ± 0.042	0.219 ± 0.033
480	0.180 ± 0.042	0.180 ± 0.033
600	-	0.164 ± 0.014
720	0.250 ± 0.046	-
1440	-	0.148 ± 0.028
Average	0.218 ± 0.039	0.149 ± 0.023

Table 4: Cumulative percent of mefenamic acid excreted in urine of male and female volunteers.

Time (min)	Male	Female
	Average ± SE	Average ± SE
60	0.083 ± 0.015	0.051 ± 0.008
120	0.277 ± 0.045	0.142 ± 0.014
180	0.515 ± 0.093	0.253 ± 0.023
240	0.753 ± 0.097	0.481 ± 0.055
360	1.096 ± 0.171	0.700 ± 0.077
480	1.276 ± 0.119	0.881 ± 0.098
600	-	1.045 ± 0.094
720	1.526 ± 0.128	-
1440	-	1.193 ± 0.112

The cumulative percent dose of mefenamic acid is given in Table 4. The value was 1.526 ± 0.128 and 1.193 ± 0.112 for male and female volunteers respectively as shown in Fig. 2.

This very low concentration of mefenamic acid in urine may be due to the formation of metabolites in greater proportion and presence of drug in free form in less proportion or body retains the drug for the longer period of time. Non-steroidal anti-inflammatory drugs (NSAIDs) are predominantly metabolized by conjugation, oxidation and hydroxylation. A small portion (1-15%) is eliminated unchanged by the kidneys [Hall *et al.* 1987].

It was concluded that excretion of free mefenamic acid in urine is little higher in male than in female volunteers. The maximum dose excreted of mefenamic acid in male at 360 minutes while in female it was at 240 minutes. In earlier study, the percent renal elimination of mefenamic acid was less than 6 percent [Wesley *et al.* 1990]. There is contradiction observed in our values and those investigated in other countries. This difference may be due to variability in dose, gender variation, and fluctuation in urine pH, environmental conditions and nutritional ingredients. Species variation may also affect the urinary excretion [Nawaz 1994].

The study on excretion of mefenamic acid provides a valuable knowledge for the rational use of drug.

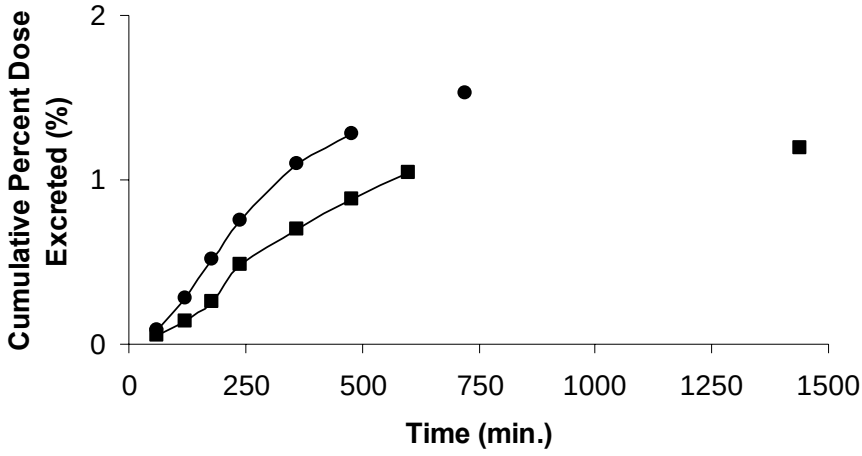


Fig. 2: Cumulative percent dose of mefenamic acid excreted (%) versus time (min).

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