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A Novel Spectrophotometric Approach for Estimation of Non-steroidal anti-inflammatory drugs (NSAIDs) in Pharmaceutical Drug formulations by Neocuproine

Ali Naser Nayef [1]*, Alaa Fark Hussain [1] and Dakhil Nassir Taha [2]
[1]Department of Chemistry, College of Science-University of Kerbala, Kerbala, Iraq.
[2]Specialized Medical Sciences, University College of Al-Amal, Karbala 56001, Iraq.
*E-mail: alielli100@gmail.com

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ABSTRACT

A novel, simple, accurate, sensitive, reproducible, economical method for spectrophotometric approach for measuring MFA in Pharmaceutical Drug formulations was successfully developed and verified. A novel analytical technique for estimating MFA 250 mg in capsule pharmaceutical formulations for different brands was established. The method suggested is based on the reduction of Cu(II)-2,9DMP complex to colored Cu(I)- 2,9DMP complex. The optimum experimental conditions have been achieved. Beer'ss Law holds true in the concentration levels 5.0-60 μg/mL with accurate molar absorptivity of 0.238 L/mol.cm at 454 nm and "detection limit" was 0.5882 μg/mL and the limit of quantification was 1.7825 μg/mL and correlation coefficient obtained has been near to one. The Effect of pH, reaction time, Buffer Solutions, Volume of Cu(II), Volume of 2,9.DMP reagent, temperature, Order of Addition, and Effect of Acid on the determination of MFA, have been examined. The proposed method has been successfully utilized for quantitative assessment of commercially produced dosage modes. Our research sought to develop a fresh, accurate, and sensitive technique for measuring MFA as a pure pharmacological product and as a hard dosage. The creation of a metal complex of MFA with a copper ion is a prerequisite for the spectrophotometric approach.

INTRODUCTION

Mefenamic Acid (MFA) it's a derivative of "2- [(2,3-dimethyl phenyl)] amino benzoic acid" as shown in (Fig.1), its anti-inflammatory non-steroidal medication belonging to the inmate class and a derivative of anthracitic acid (NSAIDs). Its mentions as painkiller medication, antipyretic, and anti-inflammatory properties. It is employed to treat mild to moderate pain. Rheumatoid arthritis is another condition for which it is suggested. MFA reduces prostaglandin synthase in a manner similar to other NSAIDs. (Yin, Hussein *et al*.,2020).
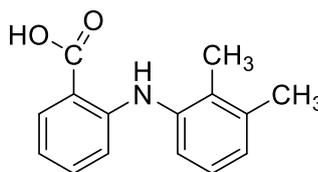

2-[(2,3-dimethylphenyl)amino]benzoic acid
Fig. 1: Chemical structure of MFA

Neocuproine is a chelating substance and heterocyclic chemical molecule $C_{14}H_{12}N_2$. The derivatives substituted at the location 2 and 9 positions are among the most researched of the substituted phenanthrolines, having been initially published in the late 19th century Similarly, the (copper (I)- Neocuproine) as a (NN ligands) with somewhat large substituents crucial. Because of its selectivity for copper(I) and potent visual absorbance of the $Cu(DMP)^{2+}$ adduct, 2,9-dimethyl-1,10-phenanthroline (NC) is typically the reagent chosen for the colourimetric measurement of copper(I) as shown in (Fig.2) (Gouda and Amin 2010).
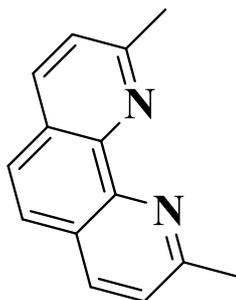
Fig. 2: 2,9-dimethyl-1,10-phenanthroline (NC)

A literature survey reveals that various analytical techniques were used for the determination of MFA such as UV spectrophotometry (Rashad and Bakir 2021), Thin layer chromatography (TLC) technique (Lillesand and Korte 2021), High-performance liquid chromatography (HPLC) technique (Jafari and haji 2020), Hydrotropic solubilization technique (Sharma and Sahoo 2021) Digital imaging colorimetry (Nepomuceno and Lemos *et al.*, 2022) Merging zone – continuous flow injection (Al-Ameer, Hashim and Taha 2022), Electrochemical sensors (Valian and Niasari,2022), Gas chromatography (Jalbani and Solangi *et al.*, 2020), Flow injection fluorometry (Hamed and Hammood 2020) Therefore, a quick, inexpensive, and selective approach is clearly required, particularly for regular quality improvement screening of pharmacological products which have MFA. However, an application of that suggested technique to the detection of MFA in a bulk tablet, and other products was that proved successful (Saleem and Alnuaimy 2021).

For the above reasons, the proposed approach's data and those from the approved method have very good agreement with both the British and American pharmacopeias as the authority's method for the determination of MFA (United States Pharmacopeial Convention 2020) (British Pharmacopoeia 2012).

Currently, the present spectrophotometric approach for determining traces of reducing agents is based on the creation of the charge transfer complex between Cu(I) and reagent 2, 9. DMP. (Almeida and Rodrigo *et al.*, 2020).

MATERIALS AND METHODS

A standard drug MFA was obtained from the state drug industry company Samara-Iraq (S.D.I), 2,9.DMP were obtained from (Merck). $Cu(NO_3)_2$ was purchased from (BDH), standardized NaOH, (Merck), and stock HCL (Chem-Lab), Deionized water was utilized for all reagent and sample dilutions, and all chemicals employed were of analytical quality.

Instrumental Condition

Shimadzuu (UVa-1700) double beam (from Japan) was used, and HANA pH meter (USA) was used for pH measurements. On the other hand, Sensitive analytical balance (four decimal places) where used from Denver Instrument (Germany). Ultrasonic Bath Cleaners from (Korea).

Sample Preparation:

A stock known concentration of 2,9-DMP 1×10^{-3} M was prepared freshly by solvation accurate amount of 0.0200 gm 2,9.DMP in 100 mL of used solvent (ethyl alcohol). NaOH 0.03 M Standardized and Prepared by dissolution 0.1600 gm NaOH in 20 mL of deionized distilled water and complete to 100 mL in a standard flask with same solvent. $Cu(NO_3)_2$ 0.01 M Solution was prepared by dissolution 0.2410gm of salt in 20 mL of distilled water and diluting it in 100 mL a standard flask with same solvent to prepare a standard solution. Buffer Solution pH=4.0 where prepared by dissolving 4.1100 gm of sodium acetate in 50 mL D.W (A), then 0.72 mL of pure acetic acid added into 125 mL D.W (B) (Saleem and Alnuaimy 2021) Standard MFA (1000 µg/mL) was prepared by dissolve 0.1000 gm MFA in 25 mL of 0.03 M standardized NaOH and complete volume up to 100 mL with same solvent, working solutions were freshly created by various subsequent dilutions.

Procedure for Dosage Forms:

Ten capsules of MEF were powdered and mixed, an exact weighted amount of powder dissolved in 25mL standardized 0.03 M NaOH, stirred, allowed to stand for 7 min, and then diluted to 100mL in a volumetric flask with the same solvent 0.03 M NaOH. This yielded the equivalent of 250.0 mg capsules. Before use, the obtained solution was filtered with Whatman filler paper no. 41 to remove any remaining or suspended materials. Working solutions were daily made through consecutive dilutions with distilled water, and they were then tested according to the suggested method.

RESULTS AND DISCUSSION

The current work presents a distinctive methodology and new reaction procedure. as well as, this method is based on the interaction between Cu(II) and the 2,9.DMP reagent to form a colourless Cu(II)-2,9.DMP complex, which enters the charge transfer reaction with MFA to form Cu(I)-2,9.DMP complex with a yellow-orange colour as shown in (Scheme 1) (ALHalboosi 2016).
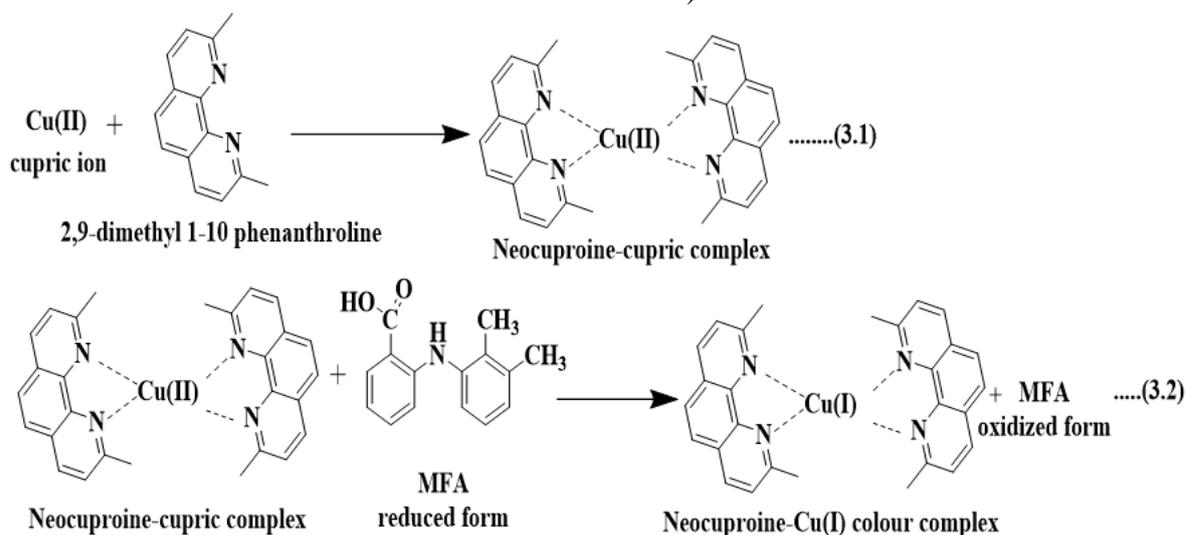
Scheme 1: Schematic representation of the suggested technique.

The formation of Cu(I) mercaptide is prevented when the metal (II)-reagent complex is considered as a reagent (Silva, Suarez and W.Toito 2018) According to the current procedure, the yellow-orange metal(I)-reagent chelate is produced.

In first few minutes following the start of the reaction, the absorbance at 454 nm changes with time as seen in Table (5). The absorbance obviously reaches a maximum after about 3 minutes and then stays constant after that. As a result (3 min) had passed since the reaction started before any absorbance measurements were taken.

It takes only a few minutes to complete the conversion of copper from copper (II) to copper (I) in the presence of 2,9.DMP.

Determination of Maximum Wavelength (λmax):

To determine the appropriate λmax, an aliquot (1mL) of the standard solution 50 μg/mL containing 50 μg of MFA was transferred to 10 mL volumetric flask, then 1mL of buffer pH4.0 and 2 mL 2,9-DMP solution 1×10^{-3} M and 1 mL of copper solution were added. The contents were mixed and completed to 10 mL with deionized distilled water, subsequently; the colored products was measured against reagent blank in the 200–1100 nm range. The maximum wavelength of MFA's absorption was observed to be 454 nm (Fig. 3). Each reagent blank displayed a negligible absorbance at the relevant λmax under the testing conditions (Kormosh and Matviychuk 2013).
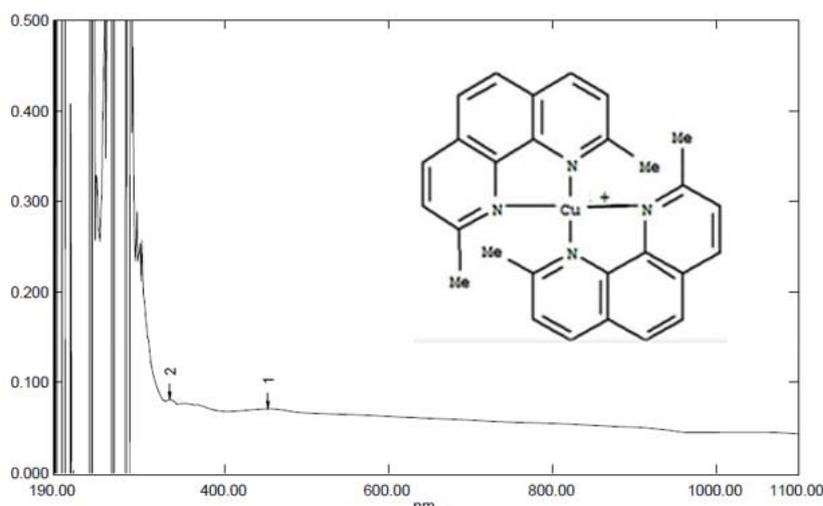
Fig. 3: Absorption spectra of reaction product (MFA =5 μg/mL) versus blank.

pH Effect:

Different volumes 0-2 mL of 0.001M standardized hydrochloric acid solution added to an aliquot of 50 μg /mL of MFA to examine the influence of pH on absorption for coloured complex. Absorption intensity for colored complex compounds was measured at 454 nm as shown in Table (1). The higher absorption intensity at pH of 4.0 is due to good intensity.

10 have been tested, 2.0 -0.25 M citric acid pH 3.0, sodium acetate pH 4.0, phosphate pH 5.0, acetate pH 6.0, 0.025 M phosphate pH 7.0, 0.02 M sodium phosphate pH 8.0, boric acid pH 9.0 and Ammonium chloride pH 10.0 (Wasito, Purnamasari and Fareza 2021) as shown in Table (2). A good sensitivity was gate at pH 4.0.

Table 1: pH effect on absorbance.

pH	Absorbance	pH	Absorbance
1	0.111	6	0.151
2	0.120	7	0.139
3	0.181	8	0.097
4	0.186	9	0.082
5	0.160	10	0.061

Table 2: Effect of buffer solution on absorbance.

Buffer	Absorbance	Buffer	Absorbance
1	0.149	6	0.136
2	0.170	7	0.119
3	0.188	8	0.079
4	0.196	9	0.028
5	0.179	10	0.024

3.3 Effect of buffer solution

Many types of buffer solution pH 1-

The resultant data from Table (2) indicate that the absorbance for the coloured complex increased when the buffer solutions

increase up to pH 4.0, and the intensity of the colored complex decreased with increasing buffer solution pH 5-10, As shown the pH 4.0 is optimum, It produces a more stable complex with stability constant $K=6.727\times10^3$

Effect of Volume of Cu(II):

The changes in Cu(II) volume on absorbance was investigated. It was observed that 1.00 mL of 1×10^{-2} M of Cu(II) gave the greatest absorption, which is strongly suggested for experimental procedures Table (3).

Table 3: Volume effects of Cu II on absorbance

Volume of Cu(II)	Absorbance
0.5 mL	0.185
1mL	0.197
1.5 mL	0.186
2 mL	0.181
2.5 mL	0.176
3 mL	0.161

Effect of Volume of 2,9.DMP Reagent:

The influence of reagent volume on the product was studied. Varying volumes of standard reagent solutions (0.5-3 mL) were added, and then read the absorbances of the solutions. However, The investigation showed that 2 mL of 2,9.DMP solution gave maximum absorbance due to its full intensity and further volume additions of reagent would form a systematic decrease in absorbance of colored product, this may be due to the creating of new species Table (4).

Table 4: Effect of volume of 2,9.DMP reagent on absorbance.

Volume of 2,9.DMP	Absorbance
0.5 mL	0.170
1 mL	0.184
1.5 mL	0.189
2 mL	0.196
2.5 mL	0.186
3 mL	0.172

Effect of Time:

Under the ideal experimental conditions established, the influence of time on stability of colored complex for various concentrations of MFA was examined. formation of colour immediately appeared after addition all reaction ingredients components and the complex's absorbance intensity held steady for at least (1 hour) (Rashid, Sarsam and Al-Sabea2017) as shown below in Table (5). The stability period is long enough to allow for the sequential performance of many measurements.

Table 5. The influence of time on the reaction product.

Time/min	Abs.	Time/min	Abs.
1	0.189	50	0.189
5	0.196	55	0.188
10	0.193	60	0.188
15	0.193	65	0.181
20	0.193	70	0.179
25	0.191	75	0.180
30	0.191	80	0.179
35	0.191	85	0.174
40	0.189	90	0.160
45	0.189		

Effect of Temperature:

The colour intensity of proposed approach was evaluated at various temperatures. The findings demonstrate that such absorbance values reaction maximum value at 25C° and remained constant up to 30C° Table (6).

Table 6: Effect of temperature on the formation of the reaction product.

Temp C°	Abs.	Temp C°	Abs.
10	0.169	55	0.154
15	0.180	60	0.142
20	0.191	65	0.130
25	0.197	70	0.111
30	0.189	75	0.090
35	0.182	80	0.071
40	0.178	85	0.066
45	0.171	90	0.059
50	0.163		

Effect of Order Addition of Reactants:

The sequence of solutions added should really be accompanied to obtain good colour intensity which gives best results.

Otherwise, a loss in colour intensity was seen, the best order of addition was (Cu+R+buffer pH4 +D) which gives better colour intensity of the produced complex which is 0.197.

Effect of Acid:

Various type of acids has been investigated, including (HCl, HCOOH, CH_3COOH, H_3PO4, HNO_3, and H_2SO_4), The mixture of 50 µg/mL drugs was added to 1.0 mL of 0.05 M standardized acids in order to determine the most efficient type of acid used at the suitable pH level. Then, 2 mL of 1×10^{-3} M 2,9.DMP solution, 1 ml of copper metal ion 1×10^{-2} M. However, the results showed that HCl was indeed the best type of acid, and 1 mL of HCl was chosen for the present research (Saleem and Alnuaimy 2021)

Effect of Excipients:

It is possible to study the effects of some foreign compounds that frequently pharmaceutical preparations by mixing 500.00 gm of various species with 10.00gm of MFA in a final volume of 25 mL as shown in Table (7). The results showed the applying suggested proposed procedure to determine MFA did not cause any interference from the examined foreign chemicals.

Table 7: excipients effect on MFA determination

Type	Abs.
glucose	0.197
lactose	0.196
dextrose	0.197
starch	0.196
sodium alyinate	0.197
sodium lauryl sulfate	0.196

Calibration Curve:

When absorbance was taken at 454 nm for 25 mL series calibrated flasks containing an increasing amount of MFA 5.0-60 µg/mL and 2 mL of 2,9.DMP, 1.0 mL cupper nitrate 0.01 M solution, 1mL buffer solution pH 4.0, The flasks were then diluted to the mark with de-ionized distilled water, mixed thoroughly, and the absorbance at 454 nm. was measured toward the blank sample of reagents. Beer's law was followed in the concentration range 5.0-60 µg/mL with a molar absorptivity of 0.238 L/mol.cm at 454 nm and a limit of detection of 0.588 µg/mL, and the correlation coefficient obtained was close to one as shown in (Fig. 4) (Wasito, Purnamasari and Fareza 2021).
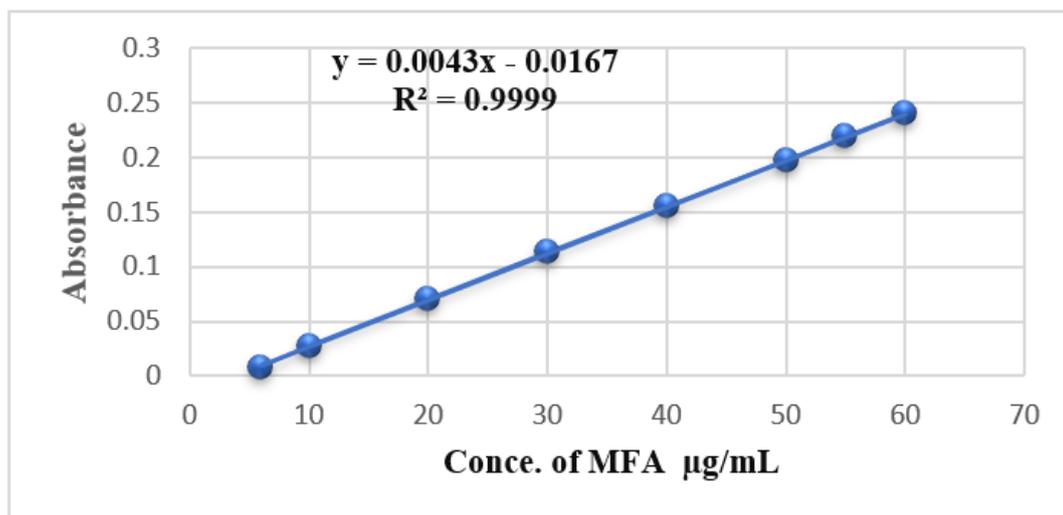
Fig. 4: Calibration graph for determination of MFA.

Table (8) shown below to illustrate the optical properties and statistical information in relation to the suggested process for the estimation of MFA in drug substances.

Table 8: Optical features and statistical information for suggested method.

Parameter	Value
Accuracy	99.623 ±1.487
Slop	0.0043
Intercept	0.0167
Linearity Range	5-60 µg/mL
Correlation coefficient	0.9999
Mean	99.623
SD	1.4871
SE Of Intercept	0.0002
SD Of Intercept	0.0007
LOD	0.5882 µg/mL
LOQ	1.7825 µg/mL

Mole Ratio Method:

Using the method of continual variations, A fixed concentration of metal ion (copper) 1×10^{-2} M was taken with increasing concentrations of the 2,9.DMP reagent 0.5-4.5×10^{-2} M and the reaction ratio between MFA. and Cu(I), [MFA / MFA +Cu(I)] was calculated as shown in (Fig.5). The findings in Figure (5) show that the ratio of MFA to Cu(I) was 1:2.
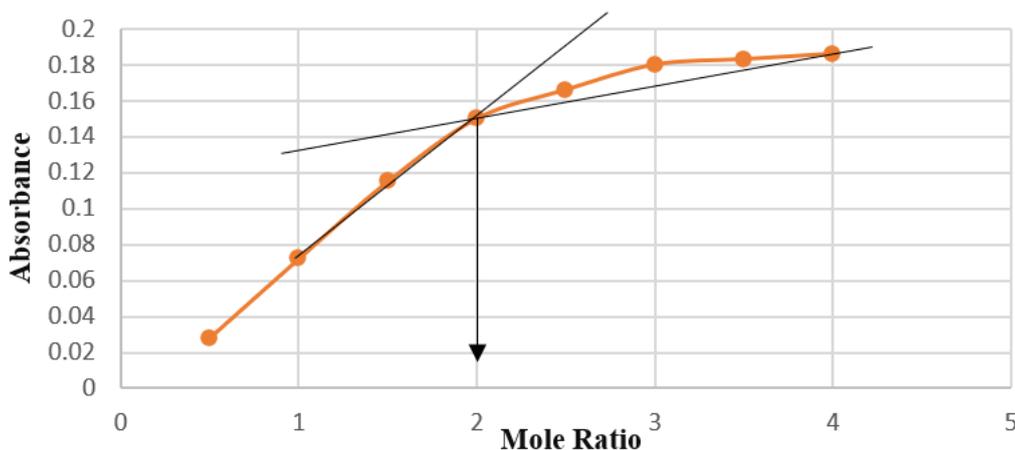
Fig. 5: Mole ratio method

Accuracy and Precision:

The calibration graph for MFA has been tested for accuracy and precision at four concentrations (10, 20,30, 40, 50 µg/mL), and the result are accurate and precise as shown in Table (9).

Table 9: Accuracy and Precision for our proposed method under optimum conditions.

Concentration of MFA µg/mL		Rec%	RSD%*	Error%*
Present	Found			
10	9.6904	96.9047	0	0
20	19.9285	99.6428	0.7246	0.0003
30	30.1666	100.5555	0	0
40	40.1666	100.4166	0.6451	0.0005
50	50.1666	100.3333	1.7255	1.9626

*n=5

Application of the Suggested Method to MFA Analyses in Pharmaceutics Formulation:

The method could be used with pharmaceutical formulations including MFA, such as Ponstidin capsule 250 mg from different brands, and mefril 250 mg MFA determination in pharmaceutical samples was accomplished successfully and with good recovery rates using the proposed method (Almeida, Rodrigo 2020) as we describe below in Table (10).

Table 10: Application of the novel framework for analysis of commercial MFA formulations in tablet dosage form (n=5).

Pharmaceutical preparation	MFA in mg	MFA measured (mg)	MFA present (µg)	MFA measured (µg)	RSD%	Error%	%Recover
Ponstidin Capsule (250 mg) N.D.I-IRAQ	250	249.5	5	4.9285	0.8247	3.3333	82.1428
			20	19.9285	0.7246	0.0003	99.6428
			40	40.1666	0.6451	0.0005	100.4166
			60	60.4047	0.2405	0.0002	100.6746
Ponstidin Capsule (250 mg) GMBH,	250	250.4	5	6.4102	0.9467	0.0005	106.8376
			20	20.3254	0.9254	0.0004	99.6725
			40	40.2564	0.4184	0.0002	100.6865
			60	60.7692	0.3219	0.0005	100.2534
Mefril (250 mg) Bangalore-India	250	250.6mg	5	5.5912	1.7543	0.0005	93.166
			20	19.9536	1.3761	0.0008	99.768
			40	40.5884	0.6024	0.0005	101.471
			60	60.1023	0.6911	0.0008	100.1705

The result above clearly shows that the developed UV technique provided good recovery beliefs in accordance with the marked quantities for every one of the analysed samples collected from many pharmaceutical companies. Furthermore, quantities analyzed within USP-specified permissible 90–110% of the MFA- selected quantity (Jarullah and Al-Hashemi 2020).

CONCLUSIONS

A novel stable and new precise accurate UV spectrometry technique for the estimation and quantification of MFA in various brands of available mefenamic acid in market MFA tablet dosage form was successfully produced. It was observed that a Cu(I)-2,9.DMP was an effective reagent for determining MFA in its purest form and in its pharmaceutical formulations.

The novel procedure relied on the production of a coloured complex (yellow-orange) between drug (mefenamic acid) and (Metal-2,9.DMP).

The testing of the samples collected from various pharmaceutical brand products demonstrated that they were in close agreement with the marked quantities and remained only within USP limits.

In industry and quality control, the proposed technique can be employed as an alternative for the rapid and routine identification of MFA in bulk material and various pharmaceutical formulations.

Declaration of Interests: The authors declare that they don't have any conflict of interest.

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