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Spectrofluorimetric analysis of menbutone in veterinary formulations: Application to residue determination in bovine meat and milk

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ABSTRACT

A simple, rapid, and sensitive spectroflourimetric method was developed for the determination of menbutone. The method is based on measuring the native fluorescence of the alkaline aqueous methanolic solution of the cited drug at its optimum excitation and emission wavelengths at λ_{em} 378 nm, upon excitation at λ_{ex} 300 nm. The method showed good linearity over the range of 0.2-2.0 µg/mL with a detection and quantitation limits of 7.25 and 24.15 ng/mL, respectively. The suggested method was successfully applied to the analysis of menbutone in its commercial veterinary formulations and the results obtained were in good agreement with those given with the manufacturer method. The method was further extended to the determination of menbutone residues in bovine meat and milk, and the results were satisfactory. The recoveries obtained were in the 98.50-102.25% range. No organic solvents were used in the extraction procedures, therefore, the proposed method can be considered as a type of 'green' chemical process.

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1. Introduction

Menbutone; 4-methoxy-γ-oxo-1-naphthalene butanoic acid (Figure 1) [1] is a specific stimulant of exocrine glands of digestive tract; digestive tonic and choloretic in bovine, ovine, porcine and equine species [2]. It helps in any course affecting the digestive system as diarrheas, anorexia and gastro-enteritis. After being injected in the body, it increases biliary, pancreatic and peptic secretion by 2 to 5 times compared with the normal levels of these secretions [3].

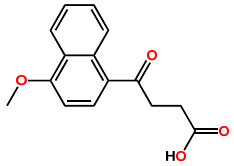

Figure 1. Chemical structure of menbutone.

In modern farming practice, drugs are used in a large scale, and are applied in animal husbandry for different reasons. They are used to prevent diseases, cure animals, or as feed additive to promote growth. In the veterinary clinic, animals digestive diseases account for about 30% of the total number of clinical diseases, and loss of appetite, indigestion, constipation and other gastrointestinal dysfunction almost concomitant diseases or secondary in most animals. Animal indigestion make animal growth stunting, production performance degradation and even death, often resulting in large economic losses, hinder the development of animal husbandry [3]. All drugs administered to milk and meat-producing animals may lead to residues in the milk and meat. There is no current legislation which establishes limits of menbutone residues in meat and milk. As a result of this lack of regulations, a zero-tolerance policy is applied for menbutone residues in baby food and formulae [4]. The use of menbutone may cause accumulation of its residues into the animal tissues and milk which ultimately find their ways into food products derived from animal origin [5]. Assays are needed to test the level of menbutone in animal products before they are brought to the market since the safety factors of it are not known.

Reviewing the literature, revealed that, few methods have been reported for the estimation of menbutone. These methods include spectrophotometry [6], HPLC-UV detection

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[7-9] and HPLC-MS [10,11]. Menbutone is very potent in its digestive activity and the regular effective doses are very small, hence, its detection in milk and meat require a highly sensitive and selective method. Spectrofluorometry has long been applied in the field of pharmaceutical analysis of many drugs [12-14] because of the higher sensitivity than is attainable in absorption spectrophotometry [15]. Analytical methods for the determination of menbutone in animal tissues and milk are, however, scarce. Therefore, it was desirable to develop a simple, sensitive and fast spectrofluorometric method that can be applied in quality control laboratories for the determination of menbutone. The results obtained were promising.

2. Experimental

2.1. Apparatus

All fluorescence measurements were made using a Perkin-Elmer UK model LS 45 Luminescence spectrometer, equipped with a 150 Watt Xenon arc lamp. The excitation and emission wavelengths were 300 and 378 nm, respectively, the slit widths were 10 nm for both excitation and emission, and the photomultiplier voltage was set to auto. Quartz 1 cm cuvette was used. Centrifugation was carried out using a TDL-60 B Centrifuge (Anke, Taiwan). Ultrasonic bath used was BHA-180 T (Abbotta, USA). Tissue homogenization was made using Tissue Master-125 with 7 mm stainless steel generator probe (Omni International, GA, USA).

2.2. Reagents and materials

All chemicals used were of analytical reagents grade, and the solvents were of HPLC grade. High purity water was used throughout the study. Pure menbutone sample was kindly supplied by Egyptian Co. for Chemicals and Pharmaceutical, Cairo, Egypt. Its purity was of 100.62% according to manufacturer method. Sodium dodecyl sulphate (SDS) was obtained from Oxford Laboratory, Mumbai (India), 1% (*w*:*v*) aqueous solution. Gelatin was obtained from ADWIC (Egypt), 1% (*w*:*v*) aqueous solution. Carboxymethyl cellulose (El-Naser Pharmaceutical Chemicals Company (ADWIC), Egypt), used as 1% (*w*:*v*) aqueous solution. Methanol and ethanol were obtained from Fisher Scientific (UK). Acetonitrile and sodium hydroxide were obtained from SD-Fine-Chem. Limited (India). 0.05 M NaOH solution was prepared in water. Sulphuric acid and hydrochloric acid were obtained from Merck (Germany). 0.05 M Sulphuric acid solutions were prepared in water. Menbutone 10% injections (B.N. 081133) each 1 mL is labeled to contain 100.0 mg menbutone, a product of the Egyptian Co. for Chemicals and Pharmaceutical, Cairo, Egypt, was purchased from local market. Bovine meat and milk were purchased from the local market.

2.3. Standard solutions

Stock solution of menbutone was prepared by dissolving 10.0 mg of the drug in 100 mL of methanol. This solution was further diluted with the same solvent to obtain a solution containing 20.0 µg/mL of menbutone. The stock solution was found to be stable for one week if stored in the refrigerator.

2.4. Construction of calibration graph

Aliquots of methanolic menbutone working standard solution were transferred into a series of 10 mL volumetric flasks to give final concentrations of 0.2-2.0 µg/mL. 4.0 mL of 0.05 M NaOH solution was added to each flask. The volume was completed with distilled water, the contents of the flasks were mixed well and the native fluorescence intensities were measured at 378 nm after excitation at 300 nm. The fluorescence intensity was plotted versus the final concentrations of the drug in µg/mL to obtain the calibration graph. Alternatively, the corresponding regression equation was derived.

2.5. Application to injections

Five menbutone 10%® injections were mixed and an aliquot of the mixed solution equivalent to 10.0 mg was transferred to a 100 mL volumetric flask and completed to volume with methanol. This solution was further diluted with the same solvent to obtain a solution claimed to contain 20.0 µg/mL of menbutone. Solutions were analyzed following the details under "Construction of calibration graph". The nominal content of the injections were obtained using the corresponding regression equation.

2.6. Preparation of bovine meat samples

The bovine meat (2.5 g) was accurately weighed, homogenized, spiked with aliquots of menbutone working standard solution (20.0 µg/mL) equivalent to 4.0-20.0 µg drug. The spiked samples were homogenized with 10 mL of methanol at 5000 rpm for 5 min; then, the homogenate was sonicated for 15 min and then centrifuged at 3,000 rpm for 5 min. The samples are re-extracted with methanol (2×5 mL), the extracts are combined, transferred to 25 mL volumetric flask, and completed to the mark with methanol. The supernatant was filtered through 0.45 µm syringe filters. The filtrate was diluted to volume with methanol. The procedure described under "Construction of calibration graph" was followed. The % recovery was calculated using the corresponding regression equation.

2.7. Preparation of bovine milk samples

Milk sample (5 mL) were transferred into 25 mL volumetric flask, spiked with aliquots of menbutone solution equivalent to 4.0-20.0 µg drug, then completed to the mark with methanol. The solution was sonicated for 2 min. The supernatant of all samples was filtered through 0.45 µm syringe filters. The filtrate was diluted to volume with methanol. The procedure described under "Construction of calibration graph" was followed. The % recovery was calculated using the corresponding regression equation.

3. Results and discussion

Literature survey revealed that only liquid chromatographic and spectrophotometric methods have been reported for the determination of menbutone. Thus, the development of spectrofluorometric methods for the determination of the cited drug in its veterinary formulations, bovine meat and milk was of interest as no such methods have been reported for the drug. The proposed method offers high sensitivity as low as 7.25 ng/mL of menbutone could be detected accurately (Table 1).

3.1. Method development

Upon exciting drug at different excitation wavelengths ranging from 230 to 390 nm in order to insure maximum sensitivity; it was found that excitation at 300 nm gave maximum fluorescence intensity at 378 nm, thus was used for its determination as shown in Figure 2. No effect of sensitizers and surfactants studied (1% aqueous gelatin, carboxymethyl cellulose and SDS on fluorescence intensity was observed; (Figure 3), thus none of them was used in this work. The effect of diluting solvent was also studied by diluting methanolic aliquots of the drug solution with 0.05 M H_2SO_4, 0.05 M HCl, methanol, ethanol, acetonitrile, water and 0.05 M NaOH; the later gave maximum fluorescence (Figure 4).

Table 1. Analytical performance data for the spectrofluorimetric determination of menbutone.

Parameter	Values
Wavelength [λ_{ex} /λ_{em}] (nm)	300/378
Linearity range (µg/mL)	0.2-2.0
Intercept (a)	-0.16
Slope (b)	139.75
Correlation coefficient (*r*)	0.9999
S.D. of residuals ($S_{y/x}$)	0.44
S.D. of intercept (S_a)	0.34
S.D. of slope (S_b)	0.28
% RSD [a]	0.36
(LOD) (ng/mL) [b]	7.25
(LOQ) (ng/mL) [c]	24.15

[a] Percentage relative standard deviation.
[b] Limit of detection.
[c] Limit of quantitation.

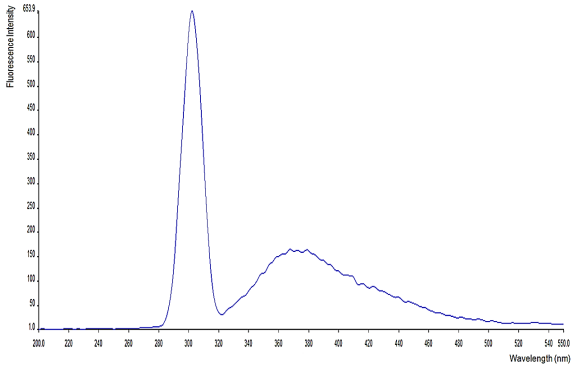

Figure 2. Excitation and emission spectra of menutone (1.2 µg/mL).

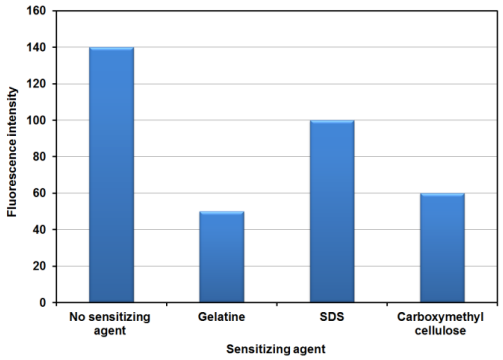

Figure 3. Effect of different sensitizing agents on fluorescence intensity of menbutone (1.0 µg/mL).

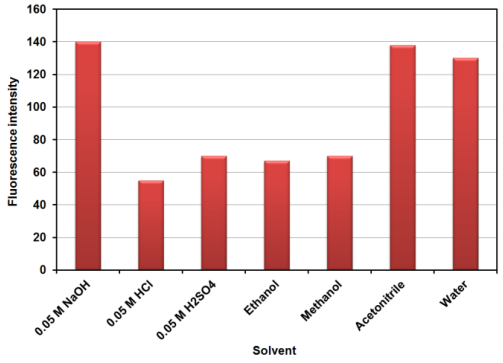

Figure 4. Effect of different solvents on the fluorescence intensity of menbutone (1.0 µg/mL).

Moreover, the effect of 0.05 M NaOH volume was also investigated using increasing volumes of 0.05 M NaOH and completing to the mark with water. It was found that maximum fluorescence intensity was obtained upon using 3 mL 0.05 M NaOH or more, lower volumes showed decreased fluorescence intensity, thus 4 mL was used (Figure 5).

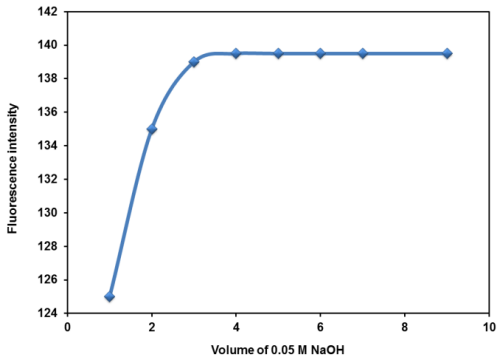

Figure 5. Effect of volume of NaOH on the fluorescence intensity of menbutone (1.0 µg/mL).

3.2. Method validation

The validity of the proposed method was assessed by studying the following parameters in accordance to ICH Q2B recommendations [16]: linearity, LOD, LOQ, accuracy and precision.

3.2.1. Linearity

The calibration graph for the determination of menbutone by the proposed method was constructed by plotting the fluorescence intensity versus its final concentration as shown in Figure 6. The graph was found to be rectilinear over the concentration range cited in Table 1. Statistical analysis of the data gave high value of the correlation coefficient (*r*) of the regression equation, small values of the standard deviation of residuals (Sy/x), of intercept (S_a), and of slope (S_b), and small value of the percentage relative standard deviation (Table 1). These data proved the linearity of the calibration graph.

3.2.2. Limit of quantitation (LOQ) and limit of detection (LOD)

The limit of quantitation (LOQ) was determined by establishing the lowest concentration of the analyte that can be measured according to ICH Q2B recommendations [16] and below which the calibration graph is non-linear and was found to be 24.15 ng/mL. The limit of detection (LOD) was determined by establishing the minimum level at which the analyte can be reliably detected; it was found to be 7.25 ng/mL as shown in Table 1.

Table 2. Assay results for the determination of menbutone in pure form by the proposed and comparison methods *.

Ranges	Proposed method			Comparison method		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Recovery	Amount taken (µg /mL)	Amount found (µg/mL)	% Recovery
0.05-2.00 µg/mL	0.2	0.201	100.5	2.5	2.452	98.08
	0.4	0.403	100.75	10.0	10.074	100.74
	0.8	0.799	99.88	25.0	24.979	99.916
	1.0	0.999	99.9			
	1.2	1.198	99.83			
	1.4	1.398	99.86			
	1.6	1.597	99.81			
	2.0	2.005	100.25			
Mean %±S.D.			100.10±0.36			99.579±1.362
t-test	1.074			(2.262)		
F-test	14.269			(19.400)		

* Each result is the average of three separate determinations. The values between parentheses are the tabulated t and F values at p = 0.05.

Table 3. Accuracy and precision data for the determination of menbutone by the proposed method *.

Range	Amount taken (µg/mL)	Intraday [a]			Interday [b]		
		Amount found±S.D. (µg/mL)	Accuracy (R%)	Precision (RSD%)	Amount found±S.D. (µg/mL)	Accuracy (R%)	Precision (RSD%)
0.2-2.0 µg/mL	0.2	0.202±0.001	101.00	0.49	0.204±0.004	102.00	1.96
	1.2	1.201±0.011	100.08	0.92	1.200±0.019	100.00	1.58
	2.0	2.011±0.019	100.55	0.95	2.017±0.023	100.85	1.14

* Each result is the average of three separate determinations.
[a] Within the day.
[b] Three consecutive days.

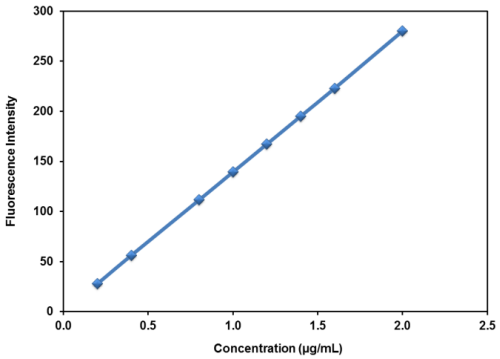

Figure 6. Calibration curve of the fluorescence intensity (λ_{em} = 378 nm, λ_{ex} = 300 nm) to concentrations of menbutone.

The values of LOQ and LOD were calculated according to the following equations:

$$LOQ = 10\ S_a/b \quad (1)$$

$$LOD = 3.3\ S_a/b \quad (2)$$

where S_a is the standard deviation of the intercept of the regression line and b is the slope of the calibration graph.

3.2.3. Accuracy

To prove the accuracy of the proposed method, the results of the assay of menbutone were compared with those of the comparison method. The comparison method is menbutone manufacturer method which depends on measuring UV-absorbance of aqueous solution of the drug at 321 nm both in pure form and injections.

Statistical analysis of the results obtained using Students' *t*-test and variance ratio *F*-test [17] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively as illustrated in Table 2.

3.2.4. Precision

Intra-day precision was achieved by determination of three concentrations of menbutone on three successive times in the same day. Inter-day precision was performed as inter-day precision but on three successive days. Small values of % RSD revealed the precision of the proposed method. The results of intra-day and inter-day precision are summarized in Table 3.

3.3. Applications

3.3.1. Application of the proposed method to the determination of menbutone in its injection solution

The developed method was applied successfully for the assay of menbutone in menbutone 10%® injection solution. The results obtained were statistically compared with those of the comparison method using *t*-test and *F*-test [17]. The results obtained were in good agreement with those obtained with the comparison method as presented in Table 4.

3.3.2. Application of the proposed method to the determination of menbutone in bovine meat and milk

The applicability of the developed procedure to determine menbutone was tested by analyzing the drug in bovine meat and milk. All samples were purchased from a local Supermarket. Methanol was used to precipitate the proteins in both milk and meat samples. Under the previously described experimental conditions, a linear relationship was established by plotting the fluorescence intensity (FI) against the corresponding drug concentration (µg/mL) in each of milk and meat. Linear regression analysis of the data gave the following regression equations:

$$FI = 136.86C - 4.59,\ r = 0.9999 \text{ (in milk)} \quad (3)$$

$$FI = 137.19C - 5.15,\ r = 0.9999 \text{ (in meat)} \quad (4)$$

The high value of the correlation coefficient indicated the good linearity of the calibration graphs. The proposed method showed satisfactory results for the determination of menbutone in bovine milk and meat. Table 5 shows the results of the analysis of menbutone determined in all samples after homogenization with extracting solution, sonication, centrifugation and filtration. The data obtained (Table 5) show satisfactory recoveries for menbutone in all samples, and the results fall in the range of 98.50-102.25 %.

Table 4. Assay results for the determination of menbutone in injection by the proposed and comparison methods*.

Parameters	Proposed method			Comparison method		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Recovery	Amount taken (µg /mL)	Amount found (µg/mL)	% Recovery
Data	0.2	0.202	101.00	2.5	2.555	102.20
	0.4	0.393	98.25	10.0	9.918	99.18
	0.6	0.602	100.33	25.0	25.026	100.11
	0.8	0.801	100.13			
	1.0	1.001	100.10			
	2.0	1.999	99.95			
Mean %±S.D.			99.96±0.92			100.49±1.55
t-test	0.667			(2.365)		
F-test	2.829			(19.300)		

* Each result is the average of three separate determinations. The values between parentheses are the tabulated t and F values at p = 0.05.

Table 5. Assay results for the determination of menbutone in bovine meat and milk by the proposed method *.

Method	Bovine meat			Bovine milk		
	Amount taken (µg/mL)	Amount found (µg/mL)	% Recovery	Amount taken (µg /mL)	Amount found (µg/mL)	% Recovery
Data	0.4	0.409	102.25	0.4	0.211	99.50
	1.0	0.985	98.50	1.0	0.482	100.30
	2.0	2.006	100.30	2.0	1.007	99.95
Mean			100.35			99.92
S.D.			1.88			0.40
% RSD			1.87			0.40

* Each result is the average of three separate determinations.

4. Conclusion

A simple, sensitive, reliable and rapid spectrofluorimetric method was developed for the determination of menbutone. The simplicity method allows the successful determination of the studied drug in its formulations and does not require elaborate treatment compared to the reported spectrophotometric and chromatographic methods. Moreover, the method was extended to the determination of menbutone residues in bovine meat and milk. It is regarded as a useful technique in the routine quality control of pharmaceutical formulations with a relatively inexpensive instrumentation. The proposed method is a non-pollutant methodology, because no organic solvents were used in the procedure, so it can therefore be considered as a type of 'green' chemical process.

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