

## Potentiometric determination of piroxicam and oxfendazole in pharmaceuticals

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Piroxicam (POC) and oxfendazole (OFA) are two widely used veterinary non-steroidal anti-inflammatory and anthelmintic medicines, respectively. Simple, precise and accurate and cost-effective methods for the determination of POC and OFZ in bulk drug and in its dosage forms have been developed and validated. The methods are based on the potentiometric titration of compound with acetic perchloric acid in glacial acetic acid medium using modified glass-saturated calomel electrode system. The methods are applicable over the ranges 1.5 - 15 mg and 7.5-15 mg, for POC and OFA, respectively. The proposed methods were successfully applied to the determination of active substances in their pharmaceutical dosage forms. A statistical comparison was made on the results of proposed methods with those obtained for reference method. The intra-day and inter-day precision and accuracy values obtained were satisfactory with RSD and RE values less than 3%. Excipients in tablets did not interfere to the assay as shown by the recovery study *via* standard addition technique with mean percentage recoveries in the range 97.8 – 100.6%.

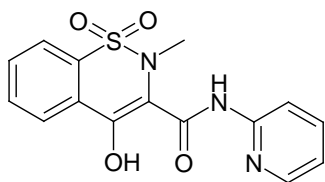
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## 1. Introduction

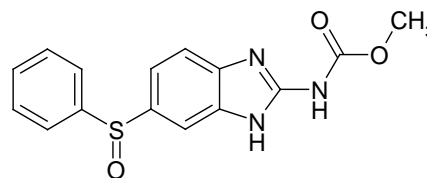
Piroxicam (POC) and oxfendazole (OFA), chemically known as 4-hydroxy-2-methyl-N-(pyridine-2-yl)-2H-1,2-benzothiazine-3-carbox-amide-1,1-dioxide (Figure 1a) and methyl-N-[6-(benzenesulfinyl)-1H-1,3-benzodiazol-2-yl]carbamate (Figure 1b), respectively, are two commonly used veterinary anti-inflammatory and anthelmintic drugs. POC has been shown to be an effective analgesic in fracture, dental, postoperative and postpartum pain. The pharmacological actions of these oxicams are related to inhibition of cyclooxygenase (Cox), a key enzyme of prostaglandine biosynthesis at the site of inflammation.<sup>1</sup> OFA, a fenbendazole sulfoxide's main metabolite, is used for protecting livestock against roundworm, strongyles and pinworms.<sup>2</sup>

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**Fig. 1a.** Structure of POC



**Fig. 1b.** Structure of OFA

Vast research is in progress to discover new drugs since many decades. Due to their inherent use many therapeutically active compounds are synthesized and hence simple quantification assay procedures are most required. POC and OFA are two commonly used therapeutics and hence simple, rapid, accurate, precise and selective procedures are required to quantify or assaying them in their pure form and in dosage forms.

Various methods have been reported for the assay of POC in both pharmaceuticals and in body fluids. These include polarographic<sup>3</sup>, capillary zone electrophoretic<sup>4</sup>, chromatographic<sup>5-11</sup>, spectrofluorimetric<sup>12,13</sup> and spectrophotometric methods<sup>14-20</sup>. In the polarographic method<sup>3</sup> carbon-paste electrode was used to perform the electrolysis in 0.05M H<sub>2</sub>SO<sub>4</sub> and buffers of pH 6 and 11 media. The capillary zone electrophoretic method<sup>4</sup> POC was quantified in dragees, suspension, suppositories, capsules, injection solutions and tablets but not validated properly. The reported chromatographic<sup>5-11</sup> and fluorimetric methods<sup>12,13</sup> are most specific for the assay of POC. However, they have demerits like sample clean up, sample pre-treatment, instrumental limitations and need of expensive instrument and skillful operator. Hence, these methods cannot be used for routine analytical work. In the spectrophotometric method reported by El-Didamony and Amin<sup>14</sup> POC was quantified by redox reaction using iron(III) and o-phenanthroline. After the oxidation of POC was completed resulted iron(II) was treated with o-phenanthroline and obtained ferriox complex was measured at 510 nm at pH 4.8. Gowda et al<sup>15</sup> was proposed an indirect procedure which involves the oxidation of POC with cerium(IV), treatment of excess cerium with methdilazine hydrochloride and measurement of red-colored product. The flow injection spectrometric method<sup>16</sup> is based on the oxidation of POC by a known excess of N-bromosuccinimide in an acidic medium, followed by a reaction of excess oxidant with chloranilic acid to bleach its purple color. The basis of El-Riesa method<sup>17</sup> is measurement of a 1:2 yellow coloured chromogen between POC and uranyl acetate at 395 nm. Sastry et al<sup>18</sup> proposed in his method that to utilization of Sevron blue 5G dye as an ion-pairing reagent. The method is based on the measurement of the absorbance of the chloroform extract of the ion-association complex formed between the drug and the dye at pH 7.0. A differential spectrophotometric method was developed by Rao et al.<sup>19</sup> The methanolic extract of POC was measured at 326 nm. In Wang's<sup>20</sup> ultra-violet spectrophotometric method POC was dissolved in 0.1 M methanolic HCl and the absorbance was measured at 334 nm.

Chromatographic<sup>21-27</sup> and radioimmunoassay<sup>28</sup> were devoted by different researchers for assaying OFA in biological materials like serum, milk, muscle and plasma. OFA was assayed before by ratio-spectra derivative spectrophotometric,<sup>29</sup> chemometric calibration<sup>30</sup> and the continuous wavelet transform<sup>31</sup> techniques in pharmaceuticals.

Though the reported spectrophotometric methods are selective for the assay of POC, they found limited application due to multistep and incomplete reactions,<sup>14-16</sup> use of hazardous and radioactive substance,<sup>17</sup> extraction steps and maintenance of stringent experimental conditions,<sup>18</sup> and measurement of absorbance at lower wavelengths<sup>19,20</sup> which is a source of photometric error.

For the assay of OFA the reported methods are not suitable to use as quality control procedures because several graphical and separation assumptions are required to be considered. Besides the reported chemometric calibration<sup>30</sup> and the continuous wavelet transform<sup>31</sup> techniques are applicable for assaying OFA in the presence of oxyclozanide.

Therefore, simple, rapid, precise and accurate, selective and cost-effective methods are required to determine POC and OFA in pure form and in dosage forms.

Potentiometric method with glass electrode can provide very accurate and sharp end points, valuable and straightforward means of assaying drug in pharmaceutical formulations because of the possibility to determine directly the active basic nitrogen containing moiety using low-cost, instrument. Simplicity, speed, compatibility and adequacy of assay procedure make the technique very attractive for the assay of pharmaceutical products.

POC and OFA are official in European Pharmacopoeia (EP)<sup>32</sup> and United States Pharmacopoeia (USP)<sup>33</sup>, respectively. Both have been quantified by non aqueous titrimetric procedures and potentiometric end point location. For each compounds the assay was performed by titrating glacial acetic acid-acetic anhydride solution of POC or formic acid and acetic anhydride solution of OFA with 0.1 N perchloric acid. These procedures involve usage of laboratory incompatible and hazardous anhydride and require 250 mg of POC or 300 mg of OFA for each titration. Hence, this is applicable for the assay in macro size samples.

Hence a smart attempt has been made to develop two simple, rapid, selective, precise and accurate, and economic methods for the assay of POC and OFA in pure and dosage forms at semi-micro level. The methods are validated according to the current ICH guidelines.<sup>34</sup>

## 2. Experimental

### 2.1 Apparatus

Elico 120 digital pH meter (Ahmedabad, India) provided with a combined glass-SCE electrode system was used to perform potentiometric titration. The KCl of the salt bridge was replaced by saturated solution of KCl in glacial acetic acid.

### 2.2 Reagents and materials

All chemicals used were of analytical reagent grade. All solutions were made in glacial acetic acid unless mentioned otherwise in titrimetric methods. The pure POC and OFA (both 99.8% pure) were kindly provided by Cipla India Ltd, Mumbai, India, received as gift and it was used. Pirox-DT and Sufentanil (both from Cipla India Ltd, Mumbai, India) POC tablets and OFA Bolus [Pharmanza (India) Pvt, Ltd] were purchased from local commercial sources.

A stock solution of (~0.1 M) perchloric acid (Merck Specialties Pvt, Ltd Mumbai, India) was diluted appropriately with glacial acetic acid to get a working solution of 5 mM and it was standardized before use.<sup>35</sup>

### 2.3 Standard drug solutions for assay

Standard solution containing 1.5 mg mL<sup>-1</sup> POC or OFA were prepared by dissolving 150 mg of pure substance in glacial acetic acid and diluting to the mark in a 100 ml calibrated flask with the same solvent.

### 2.4 General procedures

Aliquots of the standard drug solutions equivalent to 1.5 - 15 mg of POC or 7.5-15 mg of OFA was accurately measured by means of burette and transferred into a dry 100 ml beaker. The solution

was diluted to 20 ml with glacial acetic acid. Glass membrane electrode [the combined glass-SCE (modified)] was immersed into the solution. The content was titrated with standard 5 mM HClO<sub>4</sub> on magnetic stirrer. Near the equivalence point, titrant was added in 0.1 ml increments. After each addition of titrant, the solution was stirred for 20-30 s and the steady potential was noted. The addition of titrant was continued until there was no significant change in potential on further addition of titrant. The equivalence point volume was determined using titration curves (Fig. 2a and 2b). The amount of drug in the measured aliquot was calculated by using the Eq. (1) as follows,:

$$M_o = \frac{[VC]_{HClO_4} M}{n}, \quad (1)$$

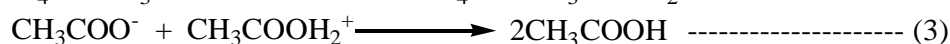
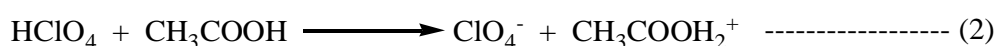
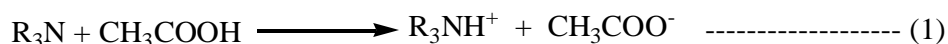
where M<sub>o</sub> is amount in milligrams of compound (relative molecular mass M) titrated with V ml of C molar HClO<sub>4</sub>. The number of moles perchloric acid reacting with each mole of the compound is denoted by n.

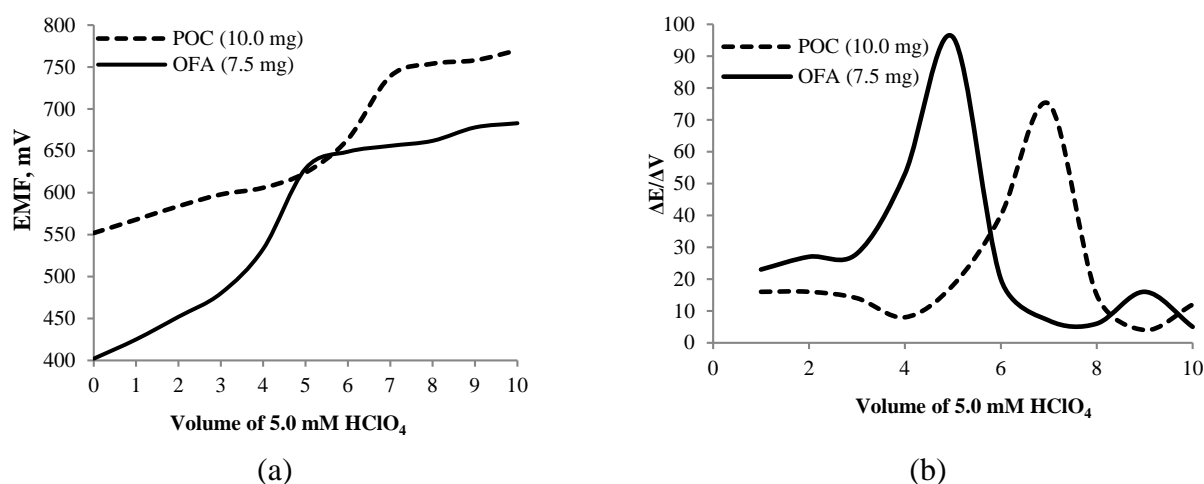
### 2.5 Procedure for tablets

Twenty tablets were weighed and pulverized. The powder equivalent to 150mg of POC/OFA was transferred into a 100 ml calibrated flask. The content was shaken with 70 ml of glacial acetic acid for about 20 min, the volume was made upto the mark with glacial acetic acid, mixed well and filtered using Whatmann No 42 filter paper. The first 10 ml portion of the filtrate was rejected and then a suitable aliquot (say 10 ml) was next subjected to analysis by the recommended procedure described above.

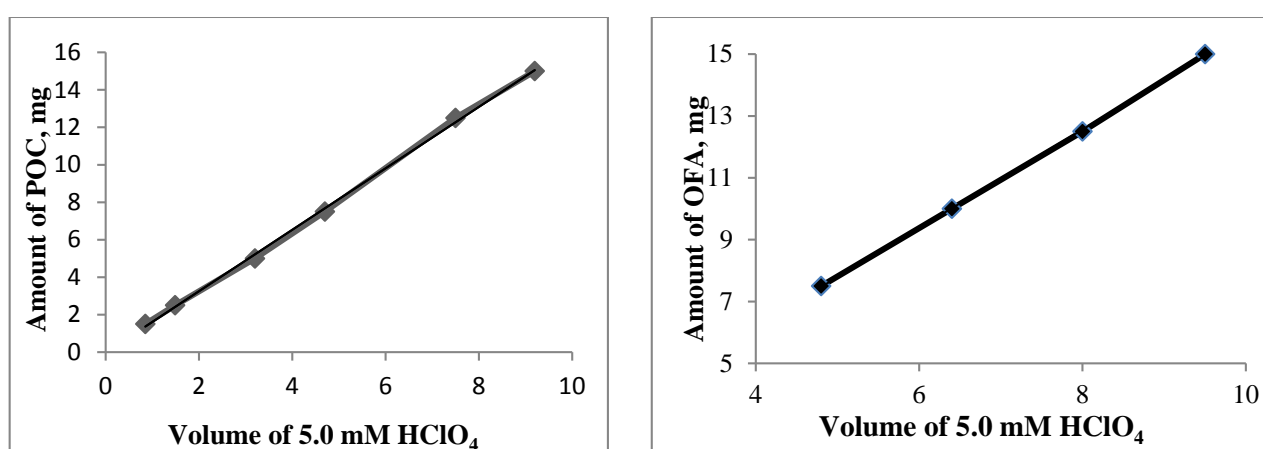
## 3. Results and discussion

POC and OFA are containing nitrogenous functional groups in their structure. The basic property of these compounds was enhanced due to the leveling effect of glacial acetic acid and titrated with perchloric acid. A steep rise in the potential was observed at the equivalence point with potentiometric end point detection (**Fig. 2**). A reaction stoichiometry of 1:1 between the titrated drug and titrant was obtained which served as the basis for calculation. Using 5 mM perchloric acid, 1.5-15 mg of POC or 7.5-15 mg of OFA was conveniently determined. Since this reaction is simply neutralization one, the relationship between the drug amount and the titration end point was correlated with potential difference. The equivalence point was determined by applying the graphical method (Figure 2). The reaction between POC/OFA and perchloric acid was found stoichiometric in the ratio 1:1 for the range of drug studied. The linear relationship between the drug and titrant was verified by the method of least squares. In which the regression coefficient values between the amounts of drug titrated and volume of titrant were found to be 0.9975 and 0.9889, for POC and OFA, respectively. The calibration plots showing the linear relationship between the quantities of drug titrated and volume of perchloric acid added are presented for both methods in Fig. 3. The following reaction scheme (1-4) is postulated for the neutralization of POC/OFA (R<sub>3</sub>N) with HClO<sub>4</sub> in acetic acid medium.





**Fig. 2.** (a) Normal titration curve and (b) First derivative titration curve



**Fig. 3.** Calibration plots showing the linear relationship between volume of HClO<sub>4</sub> consumed and amount of drug taken for titration

### 3.1 Validation of procedures

#### 3.1.1 Accuracy and precision

The accuracy and precision of the methods were checked on intra-day and inter-day basis. Three different amounts of drug within the range of study were subjected to analysis in seven and five replicates during the same day and five consecutive days. The inter-day or between-day precision of the proposed methods was performed in triplicate and for the amounts found the pooled-standard deviation was calculated. The RSD values of intra-day and inter-day studies for POC/OFA showed that the excellent precision of the methods (Table 1). The accuracy of each method was determined by the percent mean deviation from known concentration by using the Eq. (2) given as follows,

$$\%RE = \frac{D_F - D_T}{D_T} \times 100, \quad (2)$$

where  $D_F$  is milligrams of POC/OFA found and  $D_T$  is milligrams of POC/OFA subjected to assay. The calculated intra- and inter-day RE values were found in the range from 0.26 to 3.6%, and this revealed that the methods are accurate enough. These results are presented in Table 1.

**Table 1.** Accuracy and precision evaluation

Accuracy and precision	POC Taken, mg	POC Found*, mg	RE, %	RSD, %	OFA Taken, mg	OFA Found*, mg	RE, %	RSD, %
Intra-day (n = 7)	3.0	3.05	1.67	1.98	7.50	7.47	0.40	0.69
	6.0	6.11	1.83	2.11	10.0	10.04	0.40	0.89
	9.0	9.20	2.22	1.11	15.0	15.04	0.26	0.54
Inter-day (n = 5)	3.0	3.02	0.60	1.85	7.50	7.24	3.20	1.17
	6.0	6.10	1.67	1.73	10.0	10.07	0.70	2.21
	9.0	8.86	1.55	1.26	15.0	14.46	3.60	3.86

\*Mean value of n determinations

### 3.1.2 Ruggedness of the method

Method ruggedness was evaluated by performing the assay by four different analysts as well as using four different burettes. The inter-analysts RSD values lesser than 3% whereas the inter-burettes RSD for the same POC/OFA amounts was less than about 2% suggesting that the developed methods were rugged. These results are shown in Table 2.

### 3.1.3 Application

The proposed potentiometric procedures were applied to the determination of POC/OFA in their tablets. The results were statistically compared with those obtained using official EP<sup>33</sup> and UP<sup>34</sup> methods for POC and OFA, respectively. The results of the proposed methods are satisfactory when compared with those of Pharmacopeial methods and also with the label claimed. The results were compared statistically by Student's *t*-test for accuracy and by variance *F*-test for precision<sup>36</sup> with those of the reference method at 95 % confidence level. These data are summarized in Table 3. The calculated *t*- and *F*-values did not exceed the critical values at 95% confidence level for taken degrees of freedom which inferring that proposed methods are accurate and precise enough.

### 3.1.4 Recovery study

A standard addition procedure was adopted for the assessment of recovery experiments. This was done by spiking of a fixed amount of pre-analyzed drug from formulation into pure drug at three different levels, and the total was found by the proposed methods. The assay performance was checked in three replicates for each level of drug. The results, as compiled in Table 4, showed that recovery of pure drug were in the range from 98.33 to 102%, indicating that the excipients in tablets did not interfere to the assay.

**Table 2.** Method ruggedness expressed as intermediate precision (% RSD)

Variation	POC Taken, mg	RSD, %	OFA Taken, mg	RSD, %
Inter-analyst (n = 4)	3.0	1.98	7.50	1.69
	6.0	1.76	10.0	1.09
	9.0	1.21	15.0	1.33
Inter-burette (n = 4)	3.0	1.78	7.50	0.70
	6.0	0.98	10.0	1.56
	9.0	1.22	15.0	1.88

## 4. Conclusion

Simple, rapid, cost-effective and rugged methods were developed and validated for the determination of piroxicam and oxfendazole in their pure form and in tablets. The proposed methods

are more selective for the assay of POC and OFA in pure form and in dosage forms because they do not involve multistep or incomplete reactions, usage of hazardous and radioactive substance, extraction steps, maintenance of stringent experimental conditions, and measurement of absorbance at lower wavelengths. No theoretical assumptions involved in the calculation. The proposed methods could be successfully applied to the determination of 1.5-15 mg of POC or 7.5-15 mg of OFA using 5 mM solution of perchloric acid. Considering the results obtained, it is possible to assert that the methods are rapid, selective, both accurate and precise and hence suitable for the determination of drugs under investigation in their dosage forms. The proposed methods have the distinct advantages over the existing methods in terms of simplicity of technique and ease of performance and do not need expensive and highly sophisticated equipment or high-cost organic solvents which are required for HPLC analysis reported earlier. Hence, the methods can be adopted as routine analytical procedures in pharmaceutical quality control laboratories.

**Table 3.** Results of analysis of POC tablets and OFA bolus by the proposed methods and comparison of results with the reference method

Tablets	Label claim, mg/tablet	Reference Method			Proposed method			F- Value	t- Value
		%Found*	Standard deviation (S)	Variance (S <sup>2</sup> )	%Found*	Standard deviation (S)	Variance (S <sup>2</sup> )		
Pirox-DT	20	101.3	0.85	0.7225	102.8	0.96	0.9216	1.28	2.62
OFA-Bolus	2200	99.1	1.68	2.8224	97.34	1.32	1.7424	1.63	1.85

\*Mean value of five determinations

Tabulated t- and F- values at the 95 % confidence level and for four degrees of freedom are 2.77 and 6.39, respectively.

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**Table 4.** Results of recovery study

Tablet Studied	POC in tablet extract, mg	POC			Tablet Studied	OFA in Bolus extract, mg	OFA		
		Amount of pure POC added, mg	Total POC found, mg	Pure POC recovered* %			Amount of pure OFA added, mg	Total OFA found, mg	Pure OFA Recovered* %
Suganaril	4.0	2.0	6.04	102.0±0.63	OFA Bolus	3.0	3.0	5.99	99.57± 0.15
	4.0	4.0	8.05	101.3±0.72		3.0	6.0	8.95	98.33± 0.17
	4.0	6.0	9.99	99.80±1.12		3.0	9.0	11.97	99.00± 0.09

\*Mean value of three determinations

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