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Extractive Spectrophotometric Determination of Anti-Inflammatory Drug Nimesulide in Pharmaceutical Formulations and Human Plasma

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ABSTRACT

The goal of the present work is to develop simple, rapid, and sensitive extractive spectrophotometric methods for the assay of nimesulide in pharmaceutical formulations and human plasma. The methods depend on the formation of colored ion-pair complexes between nimesulide and five different reagents, bromocresol green (BCG), bromocresol purple (BCP), bromothymol blue (BTB), brilliant blue G (BBG) and methyl orange (MO) in Britton-Robinson buffer solutions of pH 2.0 - 3.5. The colored products were extracted in chloroform and absorbance measured at 412, 410, 407, 502 and 482 nm, respectively. The analytical parameters and their effects on the reported systems were investigated. The extracts are intensely colored and very stable at room temperature. The calibration graphs were linear over the concentration range of 2 - 18 μg/mL for BCG, BTB and BBG, 2 - 16 μg/mL for BCP and 2 - 14 μg/mL for MO. The stoichiometry of the reaction was found to be 1 : 1 in all cases and the conditional stability constant (K_f) of the complexes were calculated. The proposed methods were successfully extended to pharmaceutical preparations in tablet form. Excipients used as additives in commercial formulations did not interfere with the analysis. The proposed methods can be recommended for quality control and routine analysis where time, cost effectiveness and high specificity of the analytical technique are of great importance.

Key words: Nimesulide, extractive spectrophotometry, pharmaceutical, human plasma

INTRODUCTION

Nimesulide, N-(4-nitro-2-phenoxyphenyl)methanesulfonamide is a nonsteroidal anti-inflammatory drug (NSAID) that is weakly acidic (pK_a 6.5) and differs from other NSAIDs. Its chemical structure contains a sulfonanilide moiety as acidic group[(1)]. It has good anti-inflammatory, analgesic and anti-pyretic activities, and is well-tolerated by patients as demonstrated in numerous clinical trials[(2-4)]. Nimesulide is the first marketed pharmaceutical with a selective inhibition of prostaglandin synthesis via cyclo-oxygenase-2 (COX-2) [(5-7)], which results in lower toxicity in the gastrointestinal mucosa and kidney[(8)]. The safety aspects in relation to the stomach and kidney are particularly important in comparison with other NSAIDs. The chemical structure of nimesulide is shown in Figure 1.

There are several chromatographic analytical methods for the determination of nimesulide in pharmaceutical dosage forms such as LC[(9,10)], HPLC[(11-22)], HPLC/MS[(23)], HPTLC[(24,25)] and reversed-phase HPLC[(26)]. Analytical methods reported for nimesulide also include polarography[(27)], adsorptive voltammetry[(28-31)] and capillary zone electrophoresis[(32)]. A few spectrophotometric methods were reported for the quantification of nimesulide in literature, including second-order derivative UV spectroscopy[(33)] and spectrophotometry[(34-36)].

The aim of the present work is to develop simple, rapid, accurate and sensitive spectrophotometric methods for the determination of nimesulide in its pure dosage forms and human plasma.

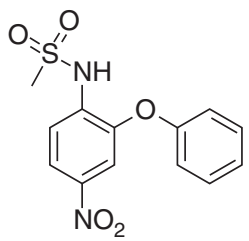

Figure 1. Chemical structure of nimesulide.

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MATERIALS AND METHODS

I. *Materials and Reagents*

All reagents used were of analytical reagent grade. Nimesulide reference standard was kindly provided by the Egyptian International Pharmaceutical Industries Co. (EIPICo), Egypt. Bromocresol green (BCG), bromocresol purple (BCP), bromothymol blue (BTB), brilliant blue G (BBG) and methyl orange (MO) were provided by BDH Chemicals Ltd., Poole, England and used without further purification. A series of Britton-Robinson (B-R) universal buffer solutions were prepared according to the standard method[37]. A stock solution of nimesulide (180 μg/mL) was prepared by dissolving 18 mg of the reference standard in a 100-mL measuring flask and diluting up to the mark with double distilled water. Working solutions of nimesulide were prepared by further dilution with water. Standard solutions of the reagents (1.0×10^{-3} M)-were prepared by dissolving accurately weighed acid dyes in a few drops of ethanol and then diluting, separately, to the mark with water in a 100-mL measuring flasks.

II. *Apparatus*

A UV-visible spectrophotometer (JASCO530 UV-Vis) with 10-mm quartz cells was used for spectrophotometric determinations. The pH measurements were performed by using a pH- meter (HI 8014, HANNA Instruments, Italy).

III. *Assay Procedure for Pure Drug*

Aliquots of nimesulide solution containing up to 180 μg/mL were transferred into a series of 1000-mL separating funnels. Buffer solutions (5.0 mL) of various pH values (2.5, 3.0, 3.0, 3.5 and 2.0) were added to various volumes (2.0, 1.8, 1.6, 1.2 and 1.0 mL) of a fixed concentration (1.0×10^{-3} M) of BCG, BCP, BTB, BBG and MO, respectively. Chloroform (10 mL) was added to each separating funnel and the contents were shaken for 2.0 min. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbances of the yellow-colored species for all reagents (except BBG, which forms a blue-colored product) were measured against a reagent blank at the values of λ_{max} (Table 1). The calibration curves for the five proposed methods were constructed by plotting the absorbance of the colored product against the final concentration of nimesulide.

IV. *Assay Procedure for Nimesulide Formulations*

Ten commercial tablets of Sulide® or Nimalox® (100 mg/tablet) were weighed and powdered. An amount of the powder equivalent to 10 mg of nimesulide was weighed and

Table 1. Quantitative parameters for determination of nimesulide

Parameter	Nimesulide				
	BCG	BCP	BTB	BBG	MO
pH	2.5	3.0	3.0	3.5	2.0
Extracting solvent	chloroform	chloroform	chloroform	chloroform	chloroform
λ_{max}	412	410	407	502	482
Molar ratio (Drug-HCl : Dye)	1 : 1	1 : 1	1 : 1	1 : 1	1 : 1
pK	5.74	5.38	6.21	5.19	5.67
Beer's law limits (μg/mL)	2.0 - 18	2.0 - 16	2.0 - 18	2.0 - 18	2.0 -14
Ringbom Range (μg/mL)	3.7 - 16.4	3.6 - 14.4	3.7 - 16.4	3.7 - 16.4	3.8 - 12.2
Molar absorptivity (L/mol/cm)	2.5×10^4	1.8×10^4	2.1×10^4	3.2×10^4	5.1×10^4
Sandell's sensitivity (ng/cm^2)	16.5	15.8	15.3	10.7	8.1
Range of error (%)	-0.66 - 0.30	-0.51 - 0.46	-0.38 - 0.46	-0.67 - 0.40	-0.54 - 0.52
Regression equation*					
Correlation coefficient *(r)*	0.9989	0.9996	0.9988	0.9991	0.9995
Intercept	0.260	0.198	0.320	0.113	0.410
Slope	0.061	0.063	0.065	0.093	0.123
t-value (2.56)**	1.19	1.36	1.22	1.63	1.67
F- value (5.05)**	2.78	2.93	2.58	3.58	3.1
LOD (μg/mL)	0.6	0.3	0.25	0.4	0.5
LOQ (μg/mL)	2.0	1.0	0.8	1.2	1.7

* A = a + bC, where C is the concentration in μg/mL.

** Values in parentheses are the theoretical values for *t*- and F- values at 95% confidence limits and five degrees of freedom.

transferred into a 100-mL volumetric flask containing about 60 mL of double distilled water. The suspended solution was shaken thoroughly for about 10 min and filtered though a Whatman filter paper No. 40 to separate insoluble matter. The remaining filtrate was diluted to the mark with double distilled water. The general procedure described above was used for the determination of nimesulide using a blank prepared in the same manner except for the pharmaceutical. A standard addition technique was also used to confirm the accuracy and precision of the methods.

V. *Assay Procedure for Human Plasma*

Human blood samples, collected in EDTA sample tubes from healthy pharmaceutical-free volunteers, were vortexed and centrifuged at 1500 rpm for 10 min to separate the plasma components. Plasma samples (1.0 mL) and aliquots of standard nimesulide solution in the concentration range of 0.06 - 0.14 mg/mL were transferred to separating funnels. A volume of 5.0 mL of buffer solutions of various pH values (2.5, 3.0, 3.0, 3.5 and 2.0) were added to various volumes (2.0, 1.8, 1.6, 1.2 and 1.0 mL) of a fixed concentration (1.0×10^{-3} M) of BCG, BCP, BTB, BBG and MO, respectively. Chloroform (10 mL) was added to each separating funnel and then the contents were shaken for 2.0 min. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate. The absorbances of the colored species for all reagents were measured against a reagent blank at the values of λ_{max} using the calibration curves for the five proposed methods.

RESULTS AND DISCUSSION

Anionic dyes like BCG form ion-association complexes with the positively charged pharmaceutical. The pharmaceutical-dye complex, with two oppositely-charged ions, behaves as a single unit held together by an electrostatic force of attraction. Therefore, nimesulide forms ion-pair complexes in acidic medium with acidic dyes such as BCG, BCP, BTB, BBG and MO. These complexes can be extracted quantitatively using chloroform. The absorption spectra of the complexes were measured between 350 and 600 nm against blank solution containing the same reagent concentration (Figure 2). The maximum absorption values (λ_{max}) of the different complexes are shown in Table 1.

I. *Optimization of the Reaction Conditions*

Optimum reaction conditions for the quantitative determination of ion-pair complexes were established via various preliminary experiments.

(I) *Effect of pH*

It was observed that the effective extraction of the complex depends on the type and pH of buffer used. The effect of pH was studied by extracting the colored complexes in the presence of various buffers, such as phthalate buffer (pH 2.22 - 3.58), acetate buffer (pH 2.05 - 5.5), and B-R buffer (pH 2.15 - 8.45). The maximum color intensities were observed in B-R buffer solutions of various acidic pH values (2.5, 3.0, 3.0, 3.5 and 2.0) with a fixed concentration (1.0×10^{-3} M) of BCG, BCP, BTB, BBG and MO, respectively (Table 1 and Figure 3). Nimesulide contains a secondary amino group, which is protonated in acidic medium. The protonated nimesulide (1.0×10^{-3} M) forms ion-pairs with anionic dyes, which are quantitatively extracted using chloroform.

(II) *Effect of Reagent Concentration*

The effect of the concentration of the reagents on the color intensities of the different complexes was examined at constant pharmaceutical concentration (1.0×10^{-3} M) using different reagent amounts at the optimum pH values. The maximum absorbance in each case was found (2.0, 1.8, 1.6, 1.2 and 1.0 mL) using a fixed concentration (1.0×10^{-3} M) of BCG, BCP, BTB, BBG and MO, respectively (Figure 4).

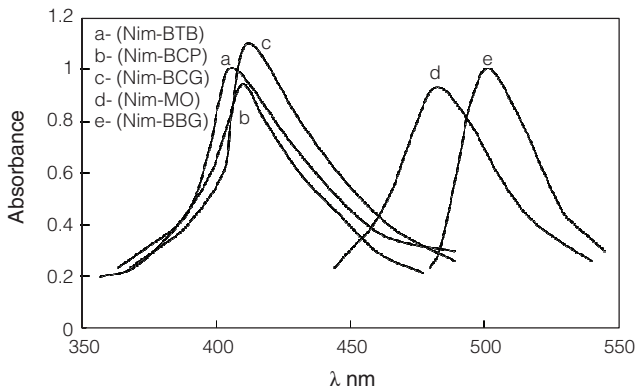

Figure 2. Absorption spectra of nimesulide-acid dye complexes extracted using chloroform.

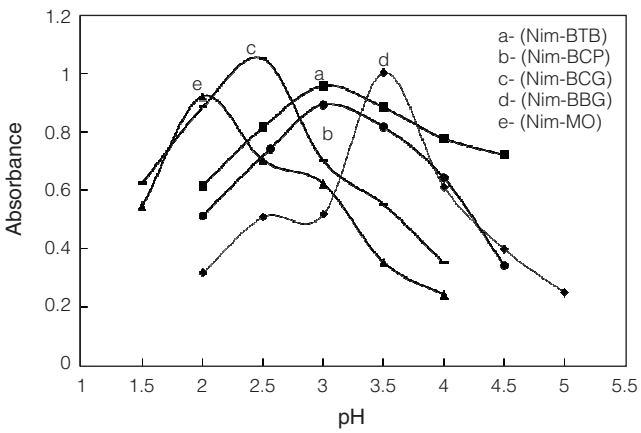

Figure 3. Effect of pH on the absorbance of nimesulide-acid dye complexes, [Nim] = [dye] = 1×10^{-3} M.

These were then used for ion-pair formations throughout the experiment.

(III) *Choice of Organic Solvent and Shaking Time*

Several organic solvents were examined for effective extraction of the colored products. The solvents included chloroform, carbon tetrachloride, dichloromethane, benzene, and toluene. Chloroform was found to be the most suitable solvent for the extraction all complexes yielding maximum absorbance intensities. A shaking time of 1.0 - 4.0 min provided constant absorbance and hence a shaking time of 1.5 min was maintained throughout the study to reach equilibrium between both phases.

(IV) *Phase Ratio*

The ratio of aqueous to organic phase was ineffective and a ratio of 1 : 1 was chosen for the extraction of the colored species. It was also observed that the order of addition of the reagents had a negligible effect on the absorbance and color of the complexes.

(V) *Effect of Temperature*

The effect of temperature on the colored complexes was studied at different temperatures (22, 27, 32, 37 and 42°C). It was found that the colored species were stable up to 42°C. At higher temperatures, the concentration of nimesulide was found to increase due to the volatile nature of the organic solvent, which resulted in an increased absorbance of the products. The colored species were found to be stable for at least 5 h at room temperature.

II. *Composition of Ion-Pair Complexes*

The pharmaceutical-reagent stoichiometric ratio as determined by Job's method (Figure 5) was found to be 1 : 1 with BCG, BCP, BTB, BBG, and MO. The extraction equilibrium can be represented as follows:

$$NimH^{+}_{(aq.)} + R^{-}_{(aq.)} \rightleftharpoons NimH^{+}.R^{-}_{(aq.)} \rightleftharpoons NimH^{+}.R^{-}_{(org.)}$$

where, $NimH^{+}$ and R^{-} represent the protonated nimesulide and the anion of the reagent, respectively. The subscripts (aq.) and (org.) refer to the aqueous and organic phases, respectively. The spectrophotometric methods that are usually applied to determine the stoichiometric ratio of the complexes, can also be used for the determination of their stability constants in solution. The values of the stability constant (pK) showed that the complexes of BTB are more stable than those of other reagents (Table 1).

III. *Method Validation*

(I) *Analytical Performance Characteristics*

The limits of the Beer-Lambert law, molar absorptivity, Sandell's sensitivity, regression equations and correlation coefficients obtained by linear square treatment of the results are given in Table 1. In order to determine the accuracy and precision of the five systems, three different concentrations of nimesulide were prepared and analyzed in six replicates and satisfactory results were obtained. Therefore, the ion-pair formations were successfully used for their determination. Percentage relative standard deviation (RSD%) and percentage relative error (RE%) were calculated as the precision and accuracy of the proposed methods, respectively (Table 2). The results of precision and accuracy obtained showed that the proposed methods have good repeatability and reproducibility. The percentage relative standard deviation (RSD%) and recoveries were found to vary over acceptable ranges (Table 2).

LOD and LOQ were calculated from 3σ and 10 σ standard deviations, respectively, which were associated with the mean of five measurements of the blank divided by the slope of the respective calibration function (Table 1).

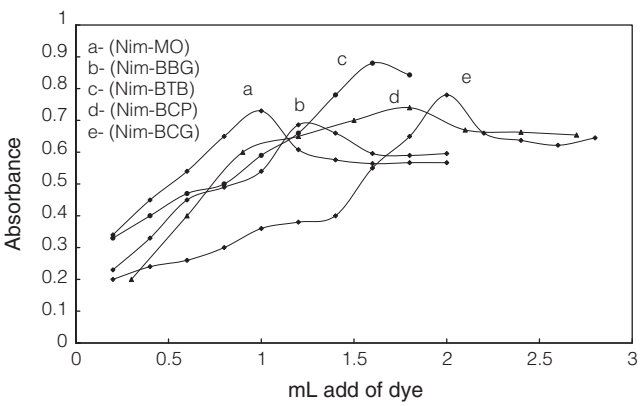

Figure 4. Effect of reagent concentration on the reaction of nimesulide with BCG, BCP, BTB, BBG and MO, [Nim] = [dye] = 1×10^{-3} M.

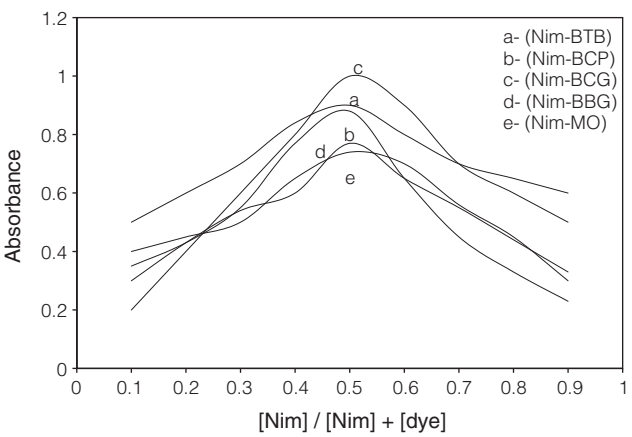

Figure 5. Job's method of continuous variation graph for the reaction of nimesulide with acid dyes BCG, BCP, BTB, BBG and MO, [Nim] = [dye] = 1×10^{-3} M.

Table 2. Evaluation of accuracy and precision for the proposed methods

Procedure	Taken μg/mL	Recovery* %	RSD %	RE %
BCG	8.0	99.56	0.128	0.34
	10.0	99.78	0.174	-0.38
	12.0	99.68	0.106	-0.57
BCP	8.0	101.04	0.107	0.45
	10.0	99.24	0.104	-0.51
	12.0	99.88	0.116	0.41
BTB	8.0	99.56	0.118	0.44
	10.0	99.77	0.110	-0.50
	12.0	99.37	0.116	-0.56
BBG	8.0	99.74	0.103	-0.35
	10.0	99.46	0.133	-0.45
	12.0	100.30	0.063	0.36
MO	8.0	99.65	0.187	0.48
	10.0	100.23	0.177	-0.68
	12.0	100.17	0.165	0.33

*Average of six determinations.

(II) *Interference Studies*

The effect of common excipients and other additives were tested for possible interferences in the assay. It was observed that talc, glucose, starch, sulfate, dextrose, acetate, phosphate, and magnesium stearate did not interfere with the determination at the levels found in dosage forms. The nimesulide content in the tablets was extracted using chloroform, which eliminated the common excipients found in the pharmaceutical formulations.

IV. *Analytical Applications*

The proposed methods have been successfully applied for the determination of nimesulide in pharmaceutical formulations. The results obtained by the proposed methods were compared with the official method (Tables 3A and 3B). For further confirmation, the standard addition method was applied to test the reliability and recovery of the proposed methods, since the ion-pair complexes are stable for at least 24 h. The high percentage recoveries indicate that the excipients in pharmaceutical dosage forms of nimesulide such as talc, glucose, starch, lactose, sulfate, dextrose, and acetate,

Table 3. Determination of nimesulide in its formulations using the proposed and official method[39]

(A)

Sample	Method	Manifested by	Taken (μg/mL)	Added (μg/mL)	Found*		Recovery (%)	RSD (%)
					Official	Proposed		
Sulide® 100 mg/tablet	BCG	Alkan Pharma S.A.E.	2.5		2.48	2.47	98.80	0.93
				2.5	5.02	4.96	99.20	0.72
				5.0	7.62	7.55	100.66	1.18
				7.5	10.10	10.07	100.70	1.02
	BCP		3.0		2.96	3.01	100.33	0.63
				3.0	5.95	6.02	100.33	0.95
				6.0	8.93	9.07	100.77	0.66
				9.0	12.05	11.96	99.66	0.78
	BTB		3.5		3.48	3.55	101.40	1.12
				3.5	6.98	7.02	100.28	0.88
				7.0	10.55	10.42	99.23	0.76
				10.5	13.98	14.03	100.21	1.08
	BBG		4.0		3.98	3.93	98.25	0.61
				4.0	8.06	7.97	99.62	0.77
				8.0	11.98	11.95	99.58	0.91
				12.0	16.10	16.05	100.31	0.67
	MO		4.5		4.47	4.48	99.55	0.86
				4.5	8.98	9.10	101.11	1.21
				9.0	13.55	13.48	99.85	0.46
				13.5	18.12	18.05	100.28	0.55

*Average of six determinations.

Table 3. Continued.
(B)

Sample	Method	Manifested by	Taken (μg/mL)	Added (μg/mL)	Found*		Recovery (%)	RSD (%)
					Official	Proposed		
Nimalox® 100 mg/tablet	BCG	SIGMA Pharmaceuticals Industries	2.5		2.48	2.47	98.80	0.93
				2.5	5.02	4.96	99.20	0.62
				5.0	7.58	7.45	99.33	0.88
				7.5	10.10	10.07	100.7	1.22
	BCP		3.0		2.96	3.01	100.33	0.73
				3.0	5.95	6.02	100.33	1.15
				6.0	8.93	9.07	100.77	0.56
				9.0	12.05	11.96	99.66	0.78
	BTB		3.5		3.42	3.55	101.4	1.15
				3.5	7.12	7.05	100.7	0.54
				7.0	10.60	10.42	99.23	0.88
				10.5	13.96	13.92	99.42	0.64
	BBG		4.0		3.93	3.98	99.50	0.71
				4.0	8.17	7.98	99.75	0.67
				8.0	11.98	11.95	99.58	0.81
				12.0	16.17	16.05	100.31	0.67
	MO		4.5		4.62	4.46	99.20	1.05
				4.5	8.94	9.08	100.9	1.22
				9.0	13.47	13.44	99.55	0.89
				13.5	17.98	18.04	100.2	0.57

*Average of six determinations.

Table 4. Recovery of nimesulide added to human plasma after ion-pair complexes formation and extraction using chloroform

Concentrations added of nimesulide (μg/mL)	Concentrations found (μg/mL) and Recovery (%)									
	BCG		BCP		BTB		BBG		MO	
	(μg/mL)	(%)	(μg/mL)	(%)	(μg/mL)	(%)	(μg/mL)	(%)	(μg/mL)	(%)
14.0	13.7	97.8	13.6	97.1	13.8	98.6	13.7	97.8	13.8	98.6
12.0	11.6	96.7	11.7	97.5	11.8	98.3	11.7	97.5	11.9	99.2
10.0	9.6	96.0	9.7	97.0	9.8	98.0	9.5	95.0	9.6	96.0
8.0	7.6	95.0	7.6	95.0	7.8	97.5	7.7	96.3	7.8	97.5
6.0	5.4	90.0	5.5	91.7	5.6	93.3	5.4	90.0	5.6	93.3
Mean ± SD		95.1 ± 1.8		95.7 ± 1.2		97.1 ± 1.0		95.3 ± 2.0		96.9 ± 1.6

phosphate, and magnesium stearate were not found to exhibit any interference in the analysis.

The monitoring of the level of nimesulide in plasma is of great importance in clinical studies because of its linear elimination pharmacokinetics and the possibility of toxicity or inadequacy of the dosage after long-term treatment. The ability of the proposed method to determine nimesulide in plasma has been appraised through spiking plasma samples with the drug at different concentration levels. It was found that the level of nimesulide in plasma could be estimated with good recoveries (Table 4) in the concentration range of 6 - 14 μg/mL, indicating that there is no interference from endogenous constituents.

The results obtained for the proposed methods were compared with those obtained using the official method with potentiometric titration[39]. The calculated Student's t-values

and F-values did not exceed the theoretical ones at 95% confidence level[38]. Therefore, there is no significant difference between the proposed and official methods.

CONCLUSIONS

The proposed methods make use of simple reagent, which an ordinary analytical laboratory can afford. The methods are sufficiently sensitive to permit determination even down to 2.0 μg/mL for pharmaceutical. The proposed method is highly reliable owing to the stability of the dye and ion-pair complexes, which are ultimately measured.

The proposed methods are simple, precise, accurate and sensitive. Therefore, they can be used for routine analysis and quality control assay of nimesulide in raw material, tablets and human plasma without interference caused by the excipients expected to be present in tablets.

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