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Validation of Titrimetric-UV Spectrophotometric Method for the Simultaneous Quantification of Paracetamol, Caffeine and Ibuprofen in Pharmaceutical Dosage Forms

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Authors' contributions

This work was carried out in collaboration among all authors. Author CAO designed the study, wrote the protocols and the first draft of the manuscript. The study was performed by authors CAO, EO and AAB. Author EO managed the literature search. Authors CAO, EO and AAB reviewed the manuscript and approved the final copy.

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ABSTRACT

Aim: The objective of this study was to develop a reliable, accurate and precise Titrimetric - UV spectrophotometric method for the assay of fixed dose combination formulations involving Paracetamol, Caffeine and Ibuprofen.
Study Design: Experimental.
Place and Duration of Study: Quality Control Department of SALOM Pharmacy Limited between June, 2015 and January, 2016.

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Methodology: The method employed an extraction of Ibuprofen from a fixed dose combination product using petroleum ether (40 – 60°C) and its evaporation followed by titration. The remaining solution was basified with 1 M NaOH and Caffeine extracted with chloroform. The extract was then evaporated and the Caffeine assayed at 273 nm. Finally, the resulting solution was diluted with distilled water and Paracetamol assayed at 257 nm. The developed method was validated as per International Conference on Harmonisation specifications [Q2 (R1)].
Results: Linearity was observed in the concentration range of 3.75 - 9 µg/ml and 4.5 - 10.8 µg/ml for Paracetamol and Caffeine respectively and a titre value range of 4.80 - 11.70 ml for Ibuprofen.
Conclusion: The results demonstrated that the method is accurate, precise, specific, and robust, hence can be suitably applied for simultaneous quantification of Paracetamol, Caffeine and Ibuprofen in laboratory prepared mixtures and in commercial preparation (capsules, caplets and tablets).

Keywords: Titrimetric; UV spectrophotometric method; paracetamol; caffeine; ibuprofen.

1. INTRODUCTION

One of the key interventions, that have been deployed to mitigate problems associated with medication adherence, is the fixed dose drug combinations (FDCs) [1]. Although the conventional approach to most treatments has been the use of single agents, FDCs have become acceptable in instances when the dosage of each ingredient meets the necessity of a defined population group and so safe combinations can be adopted. In addition to that, when the combinations have established proven advantages over single compounds administered separately [1], FDCs have become handy [2]. In pain management, it is evident that most pain conditions involve more than one underlying pain generating process. The pain is transmitted via a large number of different pathways and thus, a practical treatment approach should involve using drugs or drug combinations with different mechanisms of action and different targets [3]. Fixed-dose combination analgesic products minimizes pill burden and may require lower dosages than the individual compounds [3]. For this reason, pharmaceutical industries have developed variety of fixed combinations including agents like Paracetamol, Ibuprofen, Caffeine, Tramadol, Codeine, Oxycodone and Diclofenac [3–5]. Paracetamol is a para-aminophenol derivative with both analgesic and antipyretic properties and a weak anti-inflammatory activity [6,7]. Caffeine, a methylxanthine, is a CNS stimulant that produces a condition of wakefulness and increased mental activity [7]. It also stimulates the respiratory center, increasing the rate and depth of respiration [6]. Ibuprofen, on the other hand, is a propionic acid derivative with anti-inflammatory properties used in the management of mild to moderate pain and inflammation in conditions such as dysmenorrhoea, headache including migraine, postoperative pain, dental pain, musculoskeletal and joint disorders [6,7]. These active pharmaceutical ingredients have been widely used collectively in combination dosage forms or used individually in combination with other active pharmaceutical ingredients.

Development of analytical method for the analysis of these combination products have always been a big challenge. Liquid chromatographic analytical methods have been developed for the quantification of Paracetamol, Caffeine and Ibuprofen in combination dosage forms and/or in combination with other active pharmaceutical ingredients [5,8,9]. UV spectrophotometric analytical methods for the simultaneous quantification have been developed for Paracetamol in combination with other active pharmaceutical ingredients such as Ibuprofen [10], Aspirin [11], Dexibuprofen [12], Ibuprofen and Caffeine [13], Phenylephrine HCl and Chlorpheniramine maleate [14].

The aim of this research was to develop a reliable, accurate and precise Titrimetric-UV spectrophotometric method for the assay of fixed dose combination formulations involving Paracetamol, Caffeine and Ibuprofen. Analytical method validation was employed to access the reliability of the method [15–18]. The method was validated as per ICH guidelines [Q2 (R1)] [19].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Reference standards (Active pharmaceutical ingredients)

Paracetamol (% purity: 99.91) from Tianjin Boafa Pharmaceutical Co. Ltd., China, Ibuprofen (% purity: 99.87) form Shandong Xinhua

Pharmaceutical Co. Ltd., China and anhydrous Caffeine (% purity: 99.93) from Aartii Industries Ltd, India were employed for the study.

2.1.2 Placebo (Pharmaceutical excipients)

Sodium methylparaben (Alta Laboratories Ltd., India), Tartrazine Yellow (Vidhi Dyestuff Mfg. Ltd., India), Aerosil-200® (Evonik Industries, Netherlands), Purified *T*alc (Abhishek Organic Pvt. Ltd., India), Magnesium Stearate (Legend Industries, India), Maize starch (Riddhi Sidd Gluco Biols Ltd., India), Sodium laury Sulphate (Aarti Industries Ltd., India), Microcrystalline Cellulose (Jaya Impex, India) and Sodium Starch Glycolate (Jaya Impex, India) were also employed in the study.

2.1.3 Chemicals

Petroleum ether (40–60°C), (BDH Poole, England), Phenolphthalein (BDH Poole, England), Sodium Hydroxide pellets (BDH Poole, England) and Chloroform (Merck, Darmstadt, Germany) were employed.

2.1.4 Equipment

A double beam UV-Visible Spectrophotometer (Shimadzu, UV-1800, Japan), attached to a computer software UV probe 2.34, with a spectral width of 1 nm, wavelength accuracy of ±0.1 nm and pair of 1 cm matched quartz cells, analytical balance (OHAUS Adventurer™ Pro AV264, Switzerland), thermostat regulated water bath (HH-6, China) and Gallenkamp laboratory flask shaker (Mixer Stirrer Agitator).

2.1.5 Glassware

AGARWAL® (India), borosilicate glass volumetric flasks (Grade A), pipettes (Grade A), measuring cylinders (Grade A), conical flasks (Grade B), beakers (Grade B) and 250 ml separating funnels (Grade B).

2.1.6 Commercial samples

SABUCAP™ capsules, (Label claim: Paracetamol 325 mg, Ibuprofen 200 mg and Caffeine 30 mg) and SALO EXTRA™ caplets (Label claim: Paracetamol 325 mg, Ibuprofen 200 mg and Caffeine 30 mg) from SALOM Pharmacy Limited, Ghana. POCUMOL EXTRA™ caplets (Label claim: Paracetamol 500 mg, Ibuprofen 400 mg and Caffeine 30 mg) from POKUPHARMA Limited, Ghana, and PARABU PLUS™ capsules (Label claim: Paracetamol 325 mg, Ibuprofen 200 mg and Caffeine 30 mg) from LETAP Pharmaceuticals Limited, Ghana were purchased from local pharmaceutical retail outlets for the study.

2.2 Methods

2.2.1 Preparation of standard solutions

The standard solution of Paracetamol was prepared by accurately weighing 100 mg of Paracetamol powder, dissolving in 50 ml of 0.1 M NaOH, transferring quantitatively into 100 ml volumetric flask and diluting to the mark with the same solvent. 7.5 ml of the resulting solution was diluted to 100 ml with 0.1 M NaOH. 10 ml of the resulting solution was also pipetted and diluted to 100 ml in a volumetric flask to obtain a concentration of 7.5 µg/ml (100% concentration). The standard solution of Caffeine was prepared by accurately weighing 300 mg of Caffeine powder, dissolving in 50 ml of 0.1 M NaOH, transferring quantitatively into 100 ml volumetric flask and diluting to the mark with the same solvent. 0.3 ml of the resulting solution was diluted to 100 ml with 0.1 M NaOH. A further 10 ml of the resulting solution was diluted in a volumetric flask to 100 ml with same solvent to obtain a concentration of 9 µg/ml (100% concentration). A weight of 200 mg (100% weight) was used for Ibuprofen.

2.2.2 Analytical methods

2.2.2.1 Assay of Ibuprofen (Titrimetric)

A quantity of the formulated powder (artificial mixture of reference standards and excipients) equivalent to the filled weight of the capsule or cut weight of the caplet (oblong tablet) or tablet was accurately weighed and transferred quantitatively into a 250 ml separating funnel with 40 ml of distilled water and was shaken for 5 minutes mechanically. The mixture was extracted with five 20 ml portions of petroleum ether, (the combined aqueous layer was reserved for the analysis of Paracetamol and Caffeine) and the combined petroleum ether layer was evaporated to dryness under a current of air in a fume chamber. The residue was dissolved in 20 ml of methanol previously neutralised with phenolphthalein and titrated against a standardised 0.1 M NaOH. Each ml of standardised 0.1 M NaOH is equivalent to 20.63 mg of Ibuprofen. The content of Ibuprofen was determined using Formula 1 below:

$$\%\ \text{Cont. of Ibuprofen} = (Titre - Blank) \times \text{Factor of 0.1M NaOH} \times 20.63 \times \frac{Average\ weight}{Weight\ of\ Sample} \times \frac{100}{\textbf{Label Claim}}$$

2.2.2.2 Assay of Caffeine (UV)

10 ml of 1 M NaOH was added to the combined aqueous layer and extracted with five 30 ml of chloroform, washing each chloroform extract with same 10 ml of water, (the combined aqueous layer was reserved for the analysis of paracetamol). The combined chloroform layer was evaporated to dryness on a steam bath. The residue was dissolved in 50 ml of 0.1 M NaOH, transferred quantitatively into a 100 ml volumetric flask, diluted to volume with 0.1 M NaOH and filtered. A final concentration of 9 µg/ml was prepared using 0.1 M NaOH and the absorbance was determined at 273 nm using 0.1 M NaOH as blank. The absorbance obtained was compared with an absorbance of a standard solution of Caffeine (9 µg/ml), determined at 273 nm using distilled 0.1 M NaOH as blank and the content of Caffeine determined using Formula 2 below:

$$\%\ \text{Content of Caffeine} = \frac{\textbf{Absorbance of Sample}}{\textbf{Absorbance of Standard}} \times \frac{\textbf{Purity of Standard Sample}}{\textbf{100}} \times 100$$

2.2.2.3 Assay of Paracetamol (UV)

The reserved combined aqueous layer was transferred into a 100 ml volumetric flask, diluted to volume with 0.1 M NaOH and filtered. A final concentration of 7.5 µg/ml was prepared using 0.1 M NaOH and the absorbance was determined at 257 nm using 0.1 M NaOH as blank. The absorbance obtained was compared with an absorbance of a standard solution of Paracetamol (7.5 µg/ml), determined at 257 nm using 0.1 M NaOH as blank and the content of paracetamol was determined using Formula 3 below:

$$\%\ \text{Content of Paracetamol} = \frac{\textbf{Absorbance of Sample}}{\textbf{Absorbance of Standard}} \times \frac{\textbf{Purity of Standard Sample}}{\textbf{100}} \times 100$$

2.2.3 Validation of analytical methods

2.2.3.1 Accuracy

A sample was constituted (a total weight of 276.46 mg of the placebo weighed was spiked with known quantity of standard samples of Ibuprofen, Caffeine and Paracetamol) at 80%, 100%, 120% concentration levels and assayed as per the method stated under analytical methods respectively. Three determinations were performed under each concentration levels respectively. Results are shown in Tables 1, 2 and 3 for Ibuprofen, Caffeine and Paracetamol respectively.

2.2.3.2 Repeatability

Six standard solutions of Ibuprofen, Caffeine and Paracetamol (100% level concentrations) were prepared using different weights. Six determinations were performed. Results are shown in Tables 4, 5 and 6 for Ibuprofen, Caffeine and Paracetamol respectively.

2.2.3.3 Intermediate precision

Three samples were constituted (a total weight of 276.46 mg of the placebo weighed was spiked with 200 mg of Ibuprofen, 30 mg of Caffeine and 500 mg of Paracetamol) and assayed as per the method stated under analytical methods respectively by two different analysts on the same day. Results are shown in Tables 7, 8 and 9 for Ibuprofen, Caffeine and Paracetamol respectively.

2.2.3.4 Linearity and range

Standard solutions of Ibuprofen, Caffeine and Paracetamol over a range of 50% - 120% concentration levels were prepared using different weights. The titre values, absorbance at 257 nm and 273 nm were determined for Ibuprofen, Caffeine and Paracetamol respectively. Results are shown in Graphs 1, 2, and 3 and Table 10 for Ibuprofen, Caffeine and Paracetamol respectively.

2.2.3.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were determined graphically as per ICH guidelines [19] using the following formulae:

$$\text{LOD} = 3.3 \times \frac{\sigma}{\textbf{Slope}} \qquad \text{LOQ} = 10 \times \frac{\sigma}{\textbf{Slope}}$$

Where σ = Standard Error of intercept. Results are shown in Table 11.

2.2.3.6 Specificity

In determining the specificity of Ibuprofen, 200 mg of Ibuprofen, 30 mg of Caffeine, 500 mg of Paracetamol and 276.46 mg of the placebo were accurately weighed and assayed as per the method for Ibuprofen (section 2.2.2.1). Results are shown in Table 12.

Table 1. Accuracy for ibuprofen

% Level	Weight used (mg)	Weight obtained (mg)	% Recovery	% Mean Recovery ± SEM
	160.01	160.08	100.04	
80	160.00	159.69	99.81	99.94±0.0681
	160.02	159.97	99.97	
	200.00	199.78	99.89	
100	200.00	200.02	100.01	99.90±0.0636
	200.01	199.59	99.79	
	240.01	239.77	99.92	
120	240.03	239.93	99.96	99.97±0.0322
	240.00	240.06	100.03	

N = 3 for each % level, SEM = Standard Error of the Mean

Table 2. Accuracy for caffeine

% Level	Weight used (mg)	Weight obtained (mg)	% Recovery	% Mean Recovery ± SEM
	24.01	23.94	99.71	
80	24.03	23.99	99.83	99.87±0.1090
	24.00	24.02	100.08	
	30.00	30.04	100.13	
100	30.00	29.86	99.53	99.86±0.1764
	30.02	30.00	99.93	
	36.01	35.81	99.44	
120	36.01	35.98	99.92	99.70±0.1405
	36.00	35.91	99.75	

N = 3 for each % level, SEM = Standard Error of the Mean

Table 3. Accuracy for paracetamol

% Level	Weight used (mg)	Weight obtained (mg)	% Recovery	% Mean Recovery ± SEM
	80.01	79.92	99.89	
80	80.00	80.07	100.09	100.00±0.0702
	80.02	80.11	100.11	
	100.01	99.89	99.88	
100	100.00	99.93	99.93	99.87±0.0348
	100.01	99.82	99.81	
	120.01	120.03	100.02	
120	120.03	119.93	99.91	99.97±0.0321
	120.00	119.97	99.98	

N = 3 for each % level, SEM = Standard Error of the Mean

Table 4. Repeatability for ibuprofen

Weight used (mg)	Weight obtained (mg)	% Content	Mean ± SEM	SD	%RSD
200.02	198.23	99.11			
200.00	201.05	100.53			
200.01	197.97	98.98	99.75±0.3472	0.8504	0.850
200.00	201.90	100.95			
200.00	198.04	99.02			
200.03	199.89	99.93			

N = 6, SEM = Standard Error of the Mean, SD = Standard Deviation, RSD = Relative Standard Deviation

Table 5. Repeatability for caffeine

Weight used (mg)	Weight obtained (mg)	% Content	Mean ± SEM	SD	%RSD
30.00	30.78	101.17			
30.00	29.86	99.53			
30.01	30.02	100.03	99.56±0.4283	1.0490	1.050
30.01	29.94	100.95			
30.03	29.94	99.70			
30.00	29.44	98.13			

N = 6, SEM = Standard Error of the Mean, SD = Standard Deviation, RSD = Relative Standard Deviation

Table 6. Repeatability for paracetamol

Weight used (mg)	Weight obtained (mg)	% Content	Mean ± SEM	SD	%RSD
100.00	99.49	99.89			
100.00	100.11	100.11			
100.01	98.12	98.11	99.49±0.3982	0.9754	0.980
100.01	100.65	100.64			
100.03	98.59	98.56			
100.00	100.02	100.02			

N = 6, SEM = Standard Error of the Mean, SD = Standard Deviation, RSD = Relative Standard Deviation

Table 7. Intermediate precision for ibuprofen

Sample	Analyst 1 % content	Analyst 2 % content
1	101.38	100.35
2	99.31	102.41
3	99.31	100.35
Mean ± SEM	100.00±0.6900	101.00±0.6867
SD	1.195	1.189
%RSD	1.20	1.18

Table 8. Intermediate precision for caffeine

Sample	Analyst 1 % content	Analyst 2 % content
1	99.41	101.70
2	101.71	99.63
3	99.89	101.04
Mean ± SEM	100.30±0.7005	100.80±0.6105
SD	1.213	1.057
%RSD	1.21	1.05

For Caffeine, specificity was determined by accurately weighing 30 mg of Caffeine, 200 mg of Ibuprofen, 500 mg of Paracetamol and 276.46 mg of the placebo and assaying as per the method for Caffeine (section 2.2.2.2). Results are shown in Table 13 and Figs. 1-4.

In order to establish specificity for Paracetamol, 500 mg of Paracetamol, 30 mg of Caffeine, 200 mg of Ibuprofen and 276.46 mg of the Placebo were accurately weighed and assayed as per the method for Paracetamol (section 2.2.2.3). Results are shown in Table 14 and Figs. 5-8.

Table 9. Intermediate precision for paracetamol

Sample	Analyst 1 % content	Analyst 2 % content
1	101.59	99.97
2	99.69	100.46
3	101.32	99.21
Mean± SEM	100.90±0.5935	99.88±0.3636
SD	1.0280	0.6289
%RSD	1.02	0.63

2.2.3.7 Robustness

A sample was constituted (a total weight of 276.46 mg of the placebo weighed was spiked with 200 mg of Ibuprofen, 30 mg of Caffeine and 500 mg of Paracetamol) and assayed as per the methods stated for the ingredients respectively and variations were made to the shaking time and number of extractions. Results are shown in Tables 15, 16 and 17 for Ibuprofen, Caffeine and Paracetamol respectively.

2.2.3.8 Stability of solution

The final sample solutions obtained after the extractions were allowed to stand in the dark and the contents for Ibuprofen, Caffeine and Paracetamol determined regularly over a period of 24 hrs. Results are shown in Table 18.

Table 10. Statistical data for linearity

Parameters	Ibuprofen	Caffeine	Paracetamol
Slope ± SE	0.04911±0.0002305	504.3±0.8878	713.4±2.108
Intercept ± SE	0.1107±0.04059	-0.0002060±0.0007034	0.001487±0.001392
R^2	0.9999	0.9999	0.9999
$S_{y/x}$ (SE)	0.02988	0.0005178	0.001024
F	45370	322700	114600

Table 11. Results for LOD and LOQ

Parameters	Ibuprofen (mg)	Caffeine (µg/ml)	Paracetamol (µg/ml)
LOD	2.7275	0.0460	0.0644
LOQ	8.2651	0.1395	0.1951

Table 12. Specificity (Ibuprofen)

Sample	Titre (ml)
Ibuprofen	9.70
Caffeine	**(-)**
Paracetamol	**(-)**
Placebo	**(-)**

(-): No detection

2.2.4 Analysis of commercial samples

Four commercial samples were analysed with the validated methods and the results are shown in Table 19.

2.3 Statistical Analysis

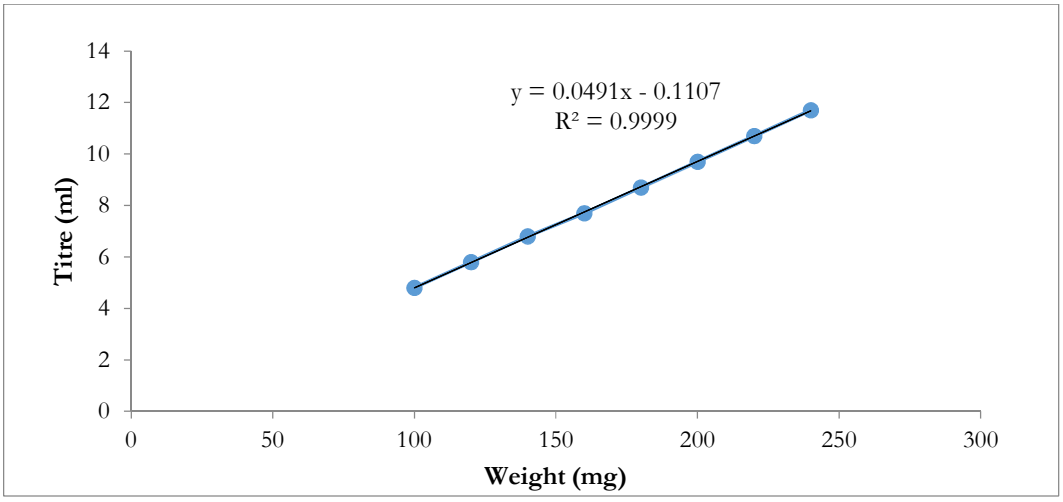
GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Data are presented as mean ± S.E.M and RSD. The values of "n" varied for each determination.

3. RESULTS AND DISCUSSION

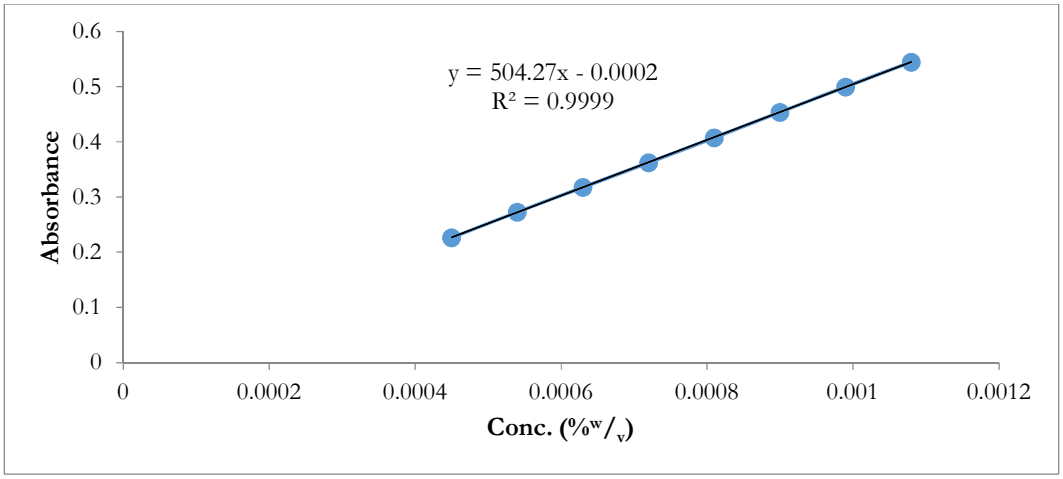
The percentage recoveries obtained were in the range of (99.90±0.0636 - 99.97±0.0322), (99.70±0.1405 - 99.87±0.1090) and (99.87±0.0348 - 100.00±0.0702) for Ibuprofen, Caffeine and Paracetamol as shown in Tables 1, 2 and 3 respectively, and were within the ICH criteria of 98-102% for accuracy. Relative Standard Deviation (RSD) values of 0.850, 1.050 and 0.980 were obtained for Ibuprofen, Caffeine and Paracetamol at 100% level concentrations as shown in Tables 4, 5 and 6 respectively which were within the ICH criteria of RSD ≤ 2% for repeatability. After the analysis of three different samples by two different analysts on the same day, the RSD obtained for Ibuprofen, Caffeine and Paracetamol by Analyst 1 were 1.20, 1.21 and 1.02 respectively and the RSD obtained for Ibuprofen, Caffeine and Paracetamol by Analyst 2 were 1.18, 1.05 and 0.63 respectively as shown in Tables 7, 8 and 9 which were within the ICH criteria of RSD ≤ 2% for intermediate precision and also there was no significant difference between the variances.

Linearity was observed in the concentration range of 3.75 - 9 µg/ml and 4.5-10.8 µg/ml for Paracetamol and Caffeine respectively and a titre value range of 4.80 - 11.70 ml for Ibuprofen. Graphs 1, 2 and 3 produced R^2 values of 0.9999 for Ibuprofen, Caffeine and Paracetamol respectively showing a good correlation between the instrument and analyte. The standard error of the slopes and intercepts, F values are shown in Tables 10. The calculated LOD for Ibuprofen, Caffeine and Paracetamol were 2.7275 mg, 0.0460 µg/ml and 0.0644 µg/ml respectively and the calculated LOQ for Ibuprofen, Caffeine and Paracetamol were 8.2651 mg, 0.1395 µg/ml and 0.1951 µg/ml respectively as shown in Table 11.

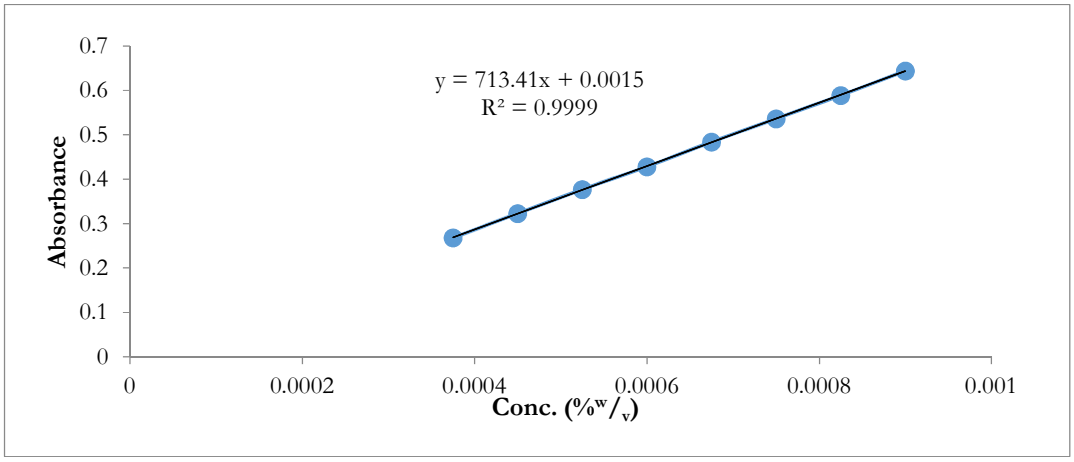
Paracetamol, Caffeine and the placebo did not interfere with the titre value of Ibuprofen as shown in Table 12 indicating that the petroleum ether used for the extraction was specific for Ibuprofen [20]. The absence of absorbance for Ibuprofen, Paracetamol and the Placebo at 273 nm indicates that they did not interfere with the absorbance of Caffeine at 273 nm as shown in Table 13 and in Figs. 1, 2, 3 and 4, indicating that the chloroform used for the extraction and the wavelength of absorption were specific for Caffeine [20]. The absorbance of Caffeine, Ibuprofen and the Placebo at 257 nm did not interfere with the absorbance of Paracetamol at 257 nm as shown in Tables 14 and in Figs. 5, 6, 7 and 8, indicating that the final aqueous solution left after the extraction contains mainly Paracetamol and the wavelength of absorption was specific for Paracetamol [20].



Graph. 1. Linearity curve for ibuprofen



Graph 2. Linearity curve for caffeine



Graph 3. Linearity curve for paracetamol

On variation of shaking time and volumes of solvents used in the extraction, the original conditions had RSD values of 0.88, 0.84 and 0.78 for Ibuprofen, Caffeine and Paracetamol respectively and the varied conditions had RSD values of 0.96, 0.75 and 1.06 for Ibuprofen, Caffeine and Paracetamol respectively as shown in Tables 15, 16 and 17, which were all within the ICH criteria of RSD ≤ 2%. For ruggedness also, there was no significant difference between the variances. On the analysis of the final solutions regularly over a period of 24 hours, RSD values of 1.10, 1.16 and 0.54 were obtained for Ibuprofen, Caffeine and Paracetamol respectively as shown in Table 18, which were also within the ICH criteria of RSD ≤ 2%, indicating that the final solutions were stable over a period of 24 hours.

The following commercial products, SABUCAP™ capsules (Label claim: Paracetamol 325 mg, Ibuprofen 200 mg and Caffeine 30 mg), SALO EXTRA™ caplets (Label claim: Paracetamol 325 mg, Ibuprofen 200 mg and Caffeine 30 mg), POCUMOL EXTRA™ caplets (Label claim: Paracetamol 500 mg, Ibuprofen 400 mg and Caffeine 30 mg) and PARABU PLUS™ capsules (Label claim: Paracetamol 325 mg, Ibuprofen 200 mg and Caffeine 30 mg), were assayed with the developed and validated method and the results are shown in Table 19.

Table 13. Specificity (Caffeine)

Sample	Absorbance @ 273 nm
Ibuprofen	**(-)**
Caffeine	0.4603
Paracetamol	**(-)**
Placebo	**(-)**

(-): No detection

Table 14. Specificity (Paracetamol)

Sample	Absorbance @ 257 nm
Ibuprofen	0.0404
Caffeine	0.0404
Paracetamol	**0.5423**
Placebo	0.0455

(-): No detection

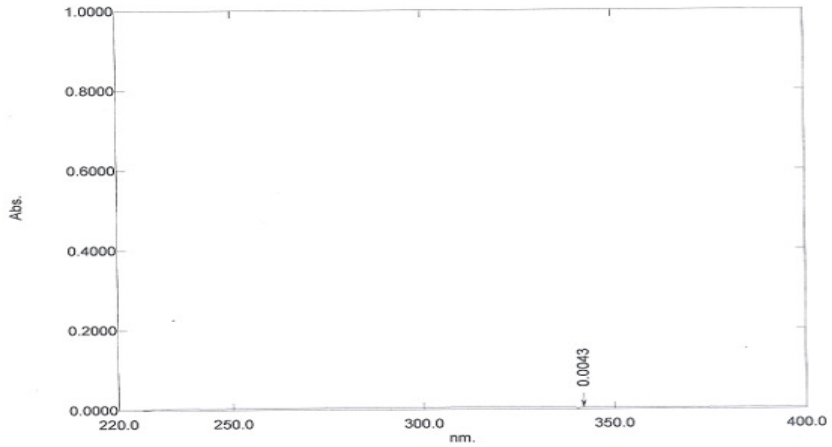


Fig. 1. Spectrum of ibuprofen @ 273 nm

Table 15. Robustness for ibuprofen

Sample	Original condition [Extraction with 5 x 20 ml petroleum ether] % Content	Varied condition [Extraction with 3 x 20 ml petroleum ether] % Content
1	99.31	99.02
2	98.61	98.12
3	100.35	100.09
Mean ± SEM	99.42±0.5055	99.10±0.5499
SD	0.8755	0.9525
%RSD	0.88	0.96

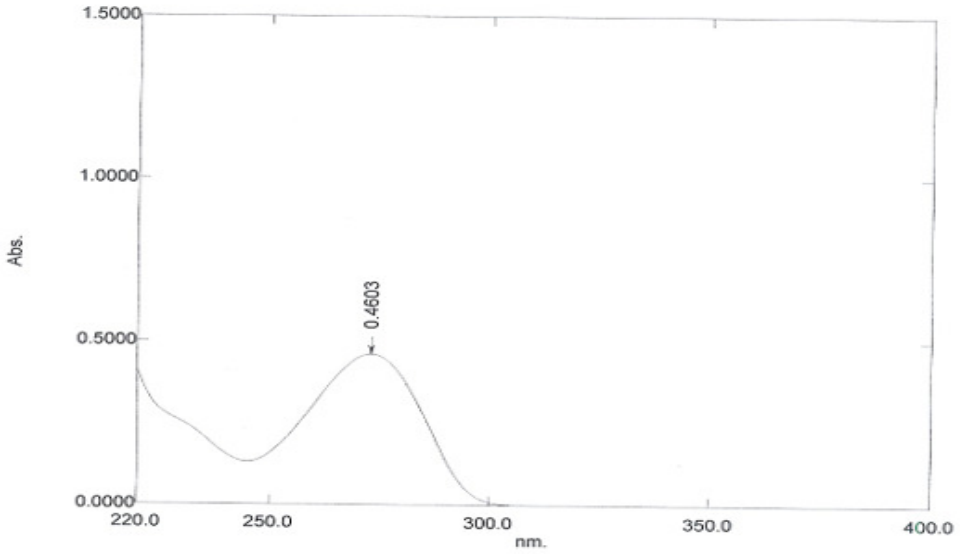


Fig. 2. Spectrum of caffeine @ 273 nm

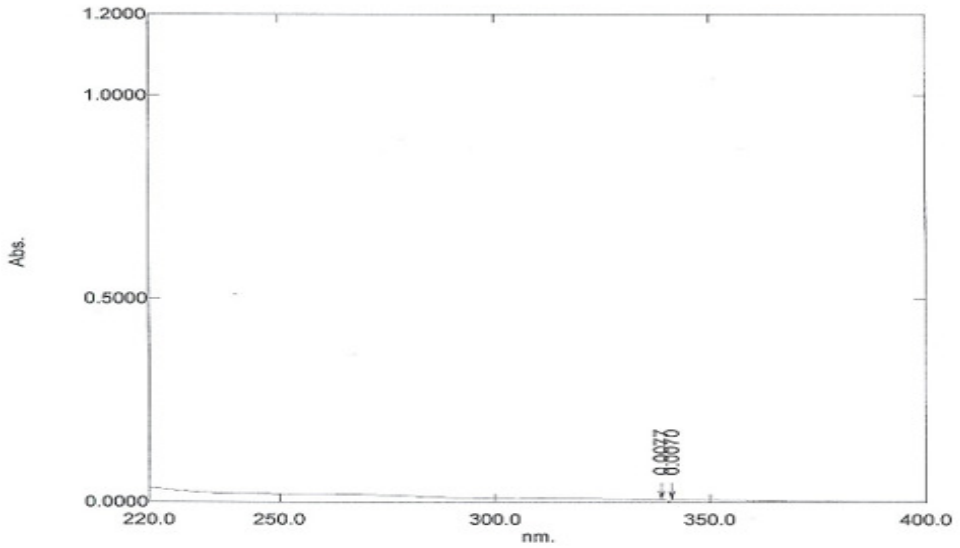


Fig. 3. Spectrum of paracetamol @ 273 nm

Table 16. Robustness for caffeine

Sample	Original condition [Extraction with 5 x 30 ml chloroform] % Content	Varied condition [Extraction with 4 x 30 ml chloroform] % Content
1	100.02	99.96
2	98.93	99.01
3	100.59	100.49
Mean ± SEM	99.85±0.4870	99.82±0.4329
SD	0.8435	0.7499
%RSD	0.84	0.75

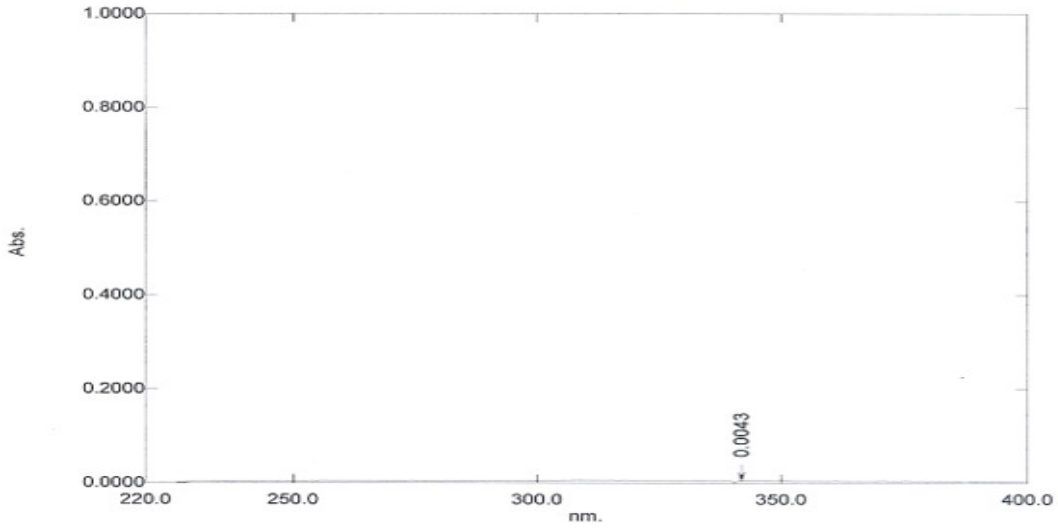


Fig. 4. Spectrum of Placebo @ 273 nm

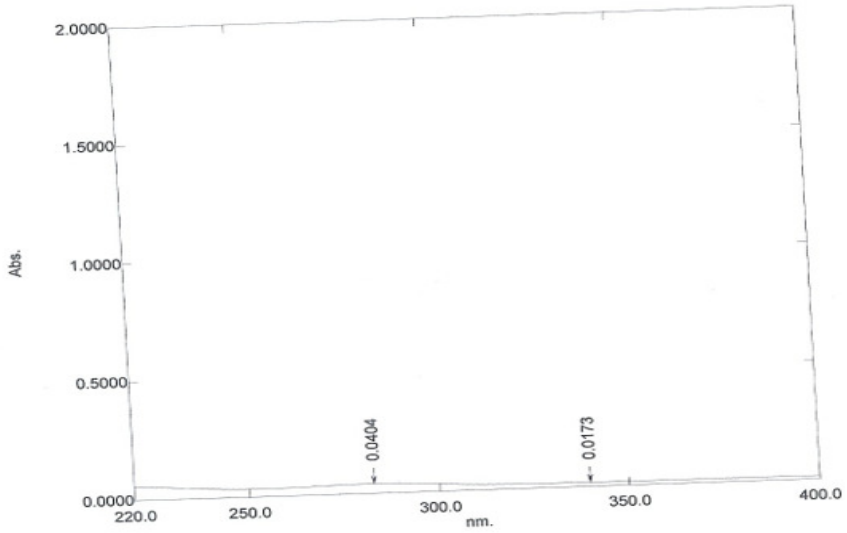


Fig. 5. Spectrum of ibuprofen @ 257 nm

Table 17. Robustness for paracetamol

Sample	Original condition [Shaking for 5 mins] % Content	Varied condition [Shaking for 3 mins] % Content
1	100.60	99.07
2	100.88	99.91
3	99.41	97.83
Mean ± SEM	100.30±0.4506	98.94±0.6041
SD	0.7805	1.046
%RSD	0.78	1.06

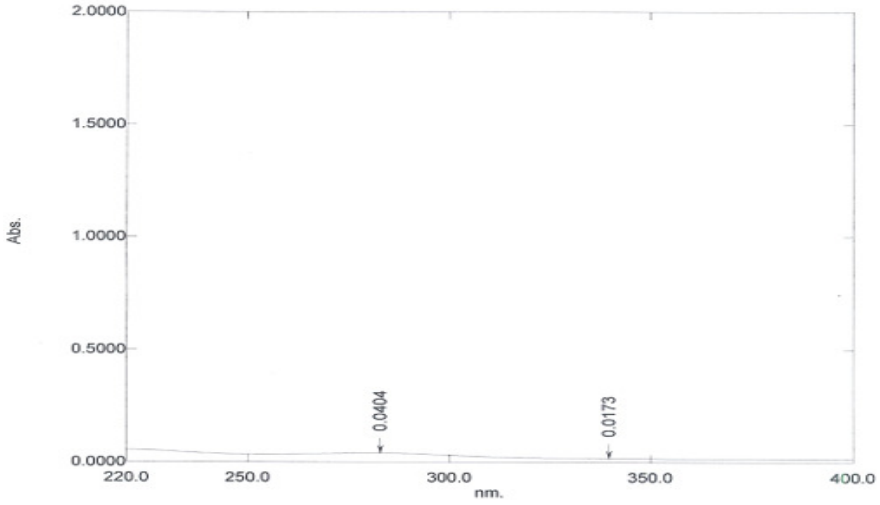


Fig. 6. Spectrum of caffeine @ 257 nm

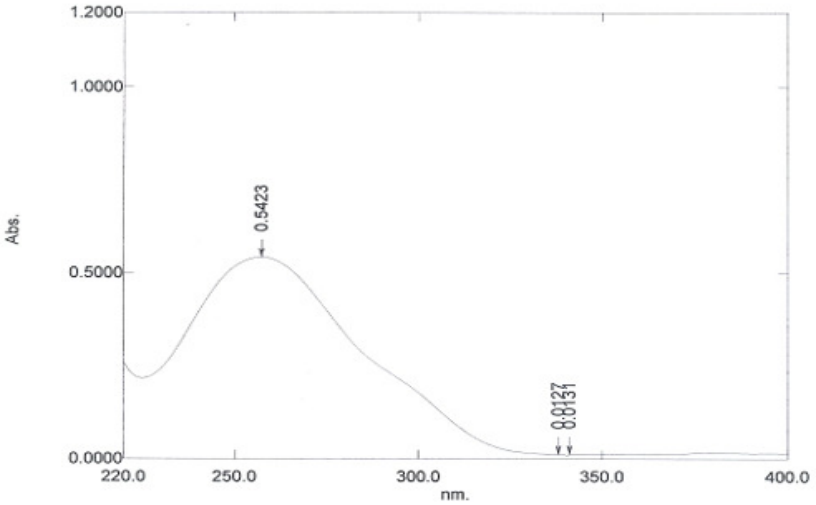


Fig. 7. Spectrum of paracetamol @ 257 nm

Table 18. Stability of solution

Time (Hrs)	Ibuprofen % content	Caffeine % content	Paracetamol % content
Initial	100.03	99.97	100.25
2	99.93	99.85	100.25
4	99.52	99.63	100.17
6	99.01	99.24	99.87
8	98.53	98.15	99.73
10	98.09	97.69	99.29
24	97.02	97.12	98.84
Mean ± SEM	98.88±0.4099	98.81±0.4318	99.77±0.2028
SD	1.085	1.142	0.5366
%RSD	1.10	1.16	0.54

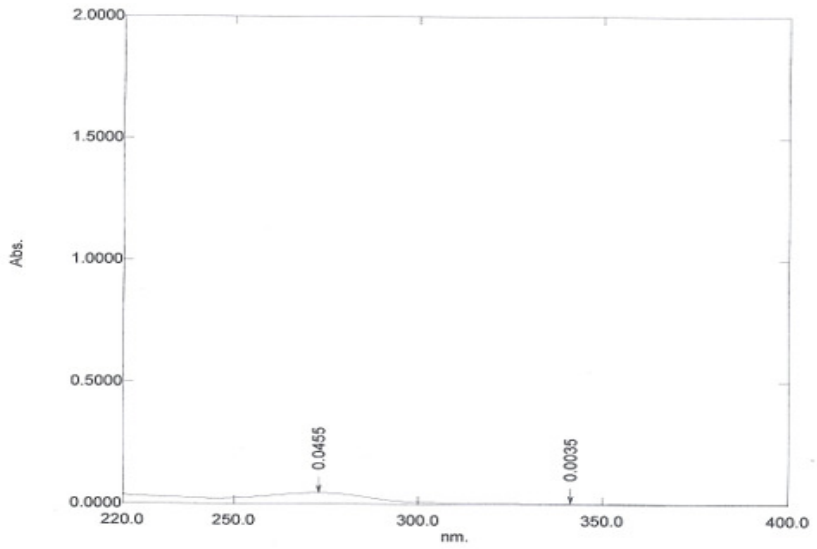


Fig. 8. Spectrum of Placebo @ 257 nm

Table 19. Commercial samples

Product	% Content		
	Ibuprofen	Caffeine	Paracetamol
SABUCAP™ capsules	100.99	100.18	100.54
SALO EXTRA™ caplets	100.76	101.03	101.49
POCUMOL EXTRA™ caplets	101.46	103.19	102.37
PARABU PLUS ™ caplets	100.75	100.21	100.58

4. CONCLUSION

The results demonstrated that the method is accurate, precise, specific and robust, hence can be suitably applied for simultaneous quantification of Paracetamol, Caffeine and Ibuprofen in laboratory prepared mixtures and in commercial preparation (capsules, caplets and tablets).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Thom S, Poulter N, Field J, Prabhakaran D, Stanton A, Grobbee DE, Bots ML, Reddy KS, Cidambi R, Bompoint S, Billot L, Rodgers A. Effects of a fixed-dose combination strategy on adherence and risk factors in patients with or at high risk of CVD: The UMPIRE randomized clinical trial. J. Am. Med. Assoc. 2013;310(9):918–929.
2. Gautam CS, Saha L. Fixed dose drug combinations (FDCs): Rational or irrational: A view point. Br. J. Clin. Pharmacol. 2008;65(5):795–796.
3. Morón Merchante I, Pergolizzi JV, van de Laar M, Mellinghoff H-U, Nalamachu S, O'Brien J, Perrot S, Raffa RB. Tramadol/Paracetamol fixed-dose combination for chronic pain management in family practice: A clinical review. ISRN Fam. Med. 2013;638469.
4. Tripathi S, Shah R, Sharma DC. Analgesic activity of fixed dose combinations of paracetamol with diclofenac sodium and paracetamol with tramadol on different pain models in healthy volunteers - A randomized double blind crossover study. J. Anaesthesiol. Clin. Pharmacol. 2012; 28(4):465–9.

5. Demiralay EC, Gümüştaş M, Canbay H. Validation of method for simultaneous determination of paracetamol and phenylephrine in pharmaceutical formulation by reversed phase liquid chromatography. Pharm. Glob. 2011;2(6): 1–4.
6. Sweetman SC. Martindale: The complete drug reference, 36th Editi. London: Pharmaceutical Press; 2009.
7. Burke A, Smyth E, FitzGerald GA. Analgesic-antipyretic agents; Pharmaco-therapy of gout," in Goodman & Gilman's The Pharmacological Basis Of Therapeutics; 2006. Electronic Copy.
8. Godse VP, Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar D, BYS. Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. Asian J. Res. Chem. 2009;2(1):37–40.
9. Battu PR, Reddy MS. RP-HPLC method for simultaneous estimation of paracetamol and ibuprofen in tablets. Asian J. Res. Chem. 2009;2(1):70–72.
10. Riddhi G, Rajashree M, Pankaj S. Development and validation of spectro-photometric methods for simultaneous estimation of ibuprofen and paracetamol in soft gelatin capsule by simultaneous equation method. Int. J Chem Tech Res. 2010;2(4):1881–1885.
11. Ghulam M, Shujaat AK, Arham S, Arshad M, Muhammad HHA, Kalsoom F, Nadia SM, Izhar H. Development of a UV-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets. Sci. Res. Essays. 2011;6(2):417–421.
12. Sohan SC, Ranjana S, Sagar BW, Amol AK. Spectrophotometric methods for simultaneous estimation of dexibuprofen and paracetamol. Asian J. Res. Chem. 2009;2(1):30–33.
13. Khoshayand MR, Abdollahi H, Shariatpanahi M, Saadatfard A, Mohammadia A. Simultaneous spectro-photometric determination of paracetamol, ibuprofen and caffeine in pharmaceuticals by chemometric methods. Spectrochim. Acta Part A. 2008;70:491–499.
14. Sawant R, Joshi R, Lanke P, Bhangale L. Simultaneous estimation and validation of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in tablets by spectrophotometric method. Asian J. Pharm. Res. Healthc. 2010;3(2):23–28.
15. Gad SC. Pharmaceutical manufacturing handbook: Regulations and quality. John Wiley and Sons, Inc., USA; 2008.
16. Swart ME, Krull IS. Analytical method development and validation. New York: Marcel Dekker, Inc., 270 Madison Avenue; 1997.
17. Huber L. Validation of Analytical methods. Agilent Technologies, Germany. 2010;7–26.
18. United States Pharmacopeia, USP 30/ NF. United States Pharmacopoeial Convention; 2006.
19. Validation of Analytical Procedures: Text and methodology Q2 (R1). Int. Conf. Harmon. Tech. Requir. Regist. Pharm. Hum. Use, ICH Harmon. Tripart. Guidel. Fed. Regist. 1997;62(96):27463–7.
20. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons. Pharmaceutical Press; 2005.

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