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NOVEL AND VALIDATED NON-AQUEOUS TITRIMETRIC METHOD FOR DETERMINATION OF PERSPECTIVE POTASSIUM-SPARING DIURETIC DRUG CANDIDATE IN PURE FORM AND PHARMACEUTICAL FORMULATION

(Kaedah Titrimetrik Bukan Akues Novel dan Ditentusahkan bagi Penentuan Penahanan Kalium-Dadah Diuretik dalam Formulasi Tulen dan Farmaseutikal)

Alexey Ivanov[1]*, Ivan Smırnov[2], Tatyana Murashko[2], Marina Trusova[1], Elena Stepanova[1]

[1]Research School of Chemistry & Applied Biomedical Sciences,
National Research Tomsk Polytechnic University, Tomsk, Russia
[2]The Faculty of Pharmacy,
Altai State Medical University, Barnaul, Russia

**Corresponding author: ivanovaa@tpu.ru*

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Abstract

4-O-β-D-(glucopyranosyloxy)-benzoic acid sodium salt (4-GBA) is promising potassium-sparing diuretic and anti-inflammatory drug. For the survey of 4-GBA as pharmaceutical substance, a durable method of its quantification is needed. In the present study, a titrimetric method for 4-GBA quantification since it is evident, very simple, fast, applicable for routine analysis and there is no need of using reference standards was developed. Aqueous titration gave unsatisfactory result. Thus, based on the weak basic properties of the 4-GBA, non-aqueous titration was chosen. Glacial acetic acid was used as a solvent, and an anhydrous solution of perchloric acid in glacial acetic acid was used as a titrant. End-point detection was carried out potentiometrically (method A) and visually with violet crystalline as an indicator (method B). Using aqueous titration, we determined pK_a of 4-GBA as 4.27. The developed non-aqueous titration method is of excellent linearity (r^2 = 0.9995 and r^2 = 0.9947 for method A and method B, respectively), accuracy (recovery from 98.8 to 101.1 % and from 98.1 to 103.2 % for method A and method B, respectively) and precision (RSD ≤ 2% for intra- and inter-day analysis). The developed method is fast, simple, cheap and is applicable for the quantitative determination of 4-GBA both in pure form, bulk and pharmaceutical formulations.

Keywords: glucosides, potassium-sparing diuretics, validation methods, non-aqueous titration, quantitative determination.

Abstrak

Garam sodium 4-O-β-D-(glukopiranosiloksi)-asid benzoik (4-GBA) merupakan penahanan diuretik kalium dan dadah anti-radang yang berkesan. Dari tinjauan 4-GBA sebagai bahan farmaseutikal, satu kaedah tahan lasak bagi kuantifikasi adalah diperlukan. Dalam kajian ini, kaedah titrimetrik bagi kuantifikasi 4-GBA telah dibangunkan kerana ia terbukti ringkas, pantas dan aplikasi mudah bagi analisis rutin dan piawai rujukan tidak diperlukan. Pentitratan akues telah memberikan hasil yang tidak memuaskan. Maka berdasarkan sifat bes lemah 4-GBA, pentitratan bukan akues telah dipilih. Asid asetik glasier telah digunakan sebagai pelarut, dan larutan asid perklorik kontang di dalam asid asetik glasier telah digunakan sebagai titran. Pengesanan titik akhir telah

dijalankan secara potentiometrik (kaedah A) dan secara visual dengan kehadiran hablur sebagai penunjuk (kaedah B). Berdasarkan pentitratan akues, nilai pKa 4-GBA ditentukan pada 4.27. Kaedah pentitratan bukan akues yang telah dibangunkan memberi nilai kelinearan baik ($r^2 = 0.9995$ dan $r^2 = 0.9947$ masing-masing bagi kaedah A dan B), ketepatan (perolehan semula dari 98.8 hingga 101.1% dan dari 98.1 hingga 103.2% masing-masing bagi kaedah A dan B) dan kejituan (RSD ≤ 2% bagi analisis intra dan inter-hari). Kaedah yang dibangunkan adalah pantas, mudah, murah dan kebolehgunaan bagi penentuan 4-GBA di dalam formulasi tulen, pukal dan farmaseutikal.

Kata kunci: glukosida, penahanan diuretik kalium, kaedah tentusahkan, pentitratan bukan akues, penentuan kuantitatif

Introduction

Diuretics are extremely important in the management of a wide range of illnesses [1-3] and considered as first-line drugs [4-5]. Among them potassium-sparing diuretics play special role in therapy since they do not increase the secretion of potassium ions into urine and maintain electrolyte balance. However, they have a number of side effects, namely, nephrolithiasis, gynecomastia, hirsutism, hyperchloremic acidosis and etc. [1, 3, 6-7]. In this context, the search for new effective and safe potassium-sparing diuretics is a critical problem of pharmaceutical chemistry.

4-O-β-D-(glucopyranosyloxy)-benzoic acid – is well-known lignin related secondary metabolite of many plants [8-12] and likely responsible for the specific biological activities of plant extracts [13]. 4-O-β-D-(glucopyranosyloxy)-benzoic acid demonstrated antioxidant [14], anti-neuroinflammatory [15] and tyrosinase inhibitory activity [13] with low toxicity in *in vitro* experiments. Recently, we demonstrated *in vivo* diuretic activity of 4-O-β-D-(glucopyranosyloxy)-benzoic acid sodium salt (4-GBA). We found that 4-GBA increases daily diuresis 1.5 and 2.5 times with chronic oral administration (7 days) at a dose of 18 μmol/kg and 54 μmol/kg, respectively. Also, 4-GBA did not increase daily clearance of sodium and potassium ions [16, 17]. We also demonstrated that 4-GBA *in vivo* has an average of two times greater anti-inflammatory activity than aspirin and did not lead to ulcerative activity in chronic oral administration for 7 days [18, 19], compared to aspirin.

The combination of biological effects allows effective treatment of chronic inflammatory and infectious urinary tract diseases. Long-term oral intake of 4-GBA will not lead to electrolyte disturbance, unlike conventional diuretics, and will not damage the digestive tract, unlike commonly used oral anti-inflammatory drugs [20, 21]. Thus, 4-GBA is a promising drug candidate for potassium-sparing diuretic and anti-inflammatory agent. For further survey, we need to develop a reliable method for quantitation of 4-GBA in raw materials, bulk and pharmaceutical formulations.

In the present paper, we developed the titrimetric methods for 4-GBA quantification. Titrimetric methods are evident, very simple, fast, applicable for routine analysis and there is no need of using reference standards [22]. The known methods for quantification of pharmaceutical substances have significant drawbacks such as high cost and multiple steps; they are time-consuming or require costly solvents [22, 23]. The proposed titrimetric method is simple with the short analytical time and good reproducibility, accuracy, durability and cost-effectiveness.

Materials and Methods

Materials

The 4-GBA was obtained from methyl ether of 4-O-β-D-(glucopyranosyloxy)-benzoic acid (ME-4-GBA) by alkaline hydrolysis (Figure 1), according to the method described [24, 25]. Before analysis, 4-GBA was dried at 80°C and 70 mbar for 8 hours. Glacial acetic acid, perchloric acid and crystal violet were obtained from Panreact (Russia). All reagents were of analytical grade. All measurements were carried out at a temperature of 25 °C. All aqueous titrations and the preparation of aqueous solutions were carried out using deionized CO_2-free water.

Instrumental

Potentiometric titration was performed on an AT-510 autotitrator (Kyoto Electronics Manufacturing Co.) equipped with a C-173 combined glass electrode and microburette (resolution 0.025 mL). Visual titration was performed involving glass burette (class A) and crystal violet as indicator (2 drops).

Hydrochloric acid (0.1 M)

A 0.1 hydrochloric acid was prepared by diluting 36% aqueous HCl (8.5 mL) with deionized water to 1 L. The solution was standardized using 0.1 M sodium carbonate (K=1.01) [26].

Perchloric acid (0.1 M)

To glacial acetic acid (900 mL) was added perchloric acid (8.2 mL), well mixed, and acetic anhydride (32 mL) was added and mixed again. The mixture was cooled to room temperature, and glacial acetic acid was added to produce 1000 mL and kept for 24 hours. The solution was standardized using 0.1 M potassium hydrogen phthalate in glacial acetic acid (K=1.09) [26].

Crystal violet indicator solution

A crystal violet indicator solution was prepared by dissolving 5 g of pure dye into 1 L of glacial acetic acid [26].

Potassium bromide solution (0.5 M)

Potassium bromide (59.5 g), was dissolved in 1 L of deionized water and mixed until complete dissolution.

Procedure of aqueous titration

Accurately weighed quantities of 4-GBA (1 g) were dissolved in deionized water (30 mL) to produce the concentration of 4-GBA 0.1 mol\L. Titration was carried out with previously standardized 0.1 M hydrochloric acid solution. End-point determination was potentiometric.

Procedure of non-aqueous titration with potentiometric determination of end-point (Method A)

Accurately weighed quantities of 4-GBA (160-240 mg, Table 1) were dissolved in glacial acetic acid (30 ml). Titration was carried out with a previously standardized 0.1 M $HClO_4$ in glacial acetic acid. The end-point was determined as the maximum on the derivative titration curve in first order. A blank experiment (in the absence of 4-GBA) was carried out in parallel.

Procedure of non-aqueous titration with visual determination of end-point (Method B)

Accurately weighed quantities of 4-GBA (160-240 mg, Table 1) were dissolved in glacial acetic acid (30 ml). Titration was carried out with a previously standardized 0.1 M $HClO_4$ in glacial acetic acid using 2 drops of crystal violet indicator solution until blue end-point.

Calculation of 4-GBA content

The content of the active substance (P) was calculated according to the Equation 1.

$$P = \frac{T \times K \times (V^0 - V^C) \times 100 \times 100}{m \times (100 - W)} \quad (1)$$

T is titer of 0.1 M $HClO_4$ in glacial acetic acid (mg/mL), K is correction factor 0.1 M $HClO_4$ in glacial acetic acid, calculated according to [26], V^0 is volume of titrant in experiment (mL), V^C is volume of titrant in blank experiment (mL), m is mass of 4-GBA (mg), W is loss on drying of 4-GBA (%). 1 mL of 0.1 M $HClO_4$ in glacial acetic acid is equivalent to 32.22 mg of 4-GBA.

The pK_a calculation

The pK_a value was determined as pH value at the half of inflection point (on the titrant volume) of 4-GBA titration curve (Figure 2A) [27]. Inflection point was determined by first and second-derivative method from titration curve (Figure 2B) [28, 29]. The measurements were carried out under the same conditions as the aqueous titration in a 0.5 M potassium bromide solution.

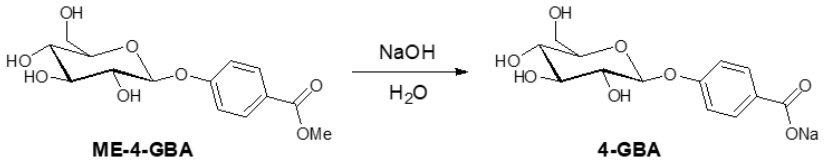

Figure 1. Preparation of 4-GBA from ME-4-GBA

Results and Discussion

The 4-GBA is a water soluble salt of a weak monobasic acid and a strong base (Figure 1). This compound in aqueous solutions is basic, therefore, acidimetry should be used for quantitative determination.

Acid-base properties of 4-GBA in aqueous solutions

The acid-base properties of 4-GBA were studied by potentiometric titration. The resulted curve is shown in Figure 2. The titrimetric analysis demonstrated one low-grade change of the pH value in a wide range from 3.75 to 1.82.

To estimate the quantitative determination of 4-GBA by the titrimetric method in an aqueous solution we calculated the negative logarithm of the dissociation constant (pK_a). There is a wide range of techniques for determining pK_a value, for example, potentiometry, conductometry, calorimetry, nuclear magnetic resonance (NMR), electrophoresis, high-performance liquid chromatography (HPLC), ultraviolet–visible spectroscopy and others [27]. We chose potentiometry for determination of pK_a value because this method is fast, simple, universal, cheap and highly precise [27, 28]. The change of solution ionic strength may influence pK_a determination [27]. Thus, we carried out pK_a measurements in 0.5 N potassium bromide solution in order to maintain constant ionic strength. Optimal concentrations of 4-GBA and hydrochloric acid solutions were determined as 0.1 N [30].

Second-derivative method was chosen for inflection point determination since it is applicable for weak bases and acids and provide accurate determination in case of titration curve has no sharp pH jump. This method is based on plot $\Delta^2pH/\Delta V^2$ vs. volume of titrant (V) (Figure 2b). The inflection point is where $\Delta^2pH/\Delta V^2$ equals 0 [29]. The equivalent titrant volume was 30 mL (Figure 2a). pK_a value was determined as pH value at the point corresponding to half of the titration (15 mL) (Figure 2a). Calculated according to this method 4-GBA $pK_a = 4.27$ indicates that 4-GBA is a very weak base.

Referring to the titration curves (Figure 2) and pK_a value of 4-GBA, the aqueous titration method for quantitative determination is of insufficient accuracy. The maximum of fist-derivative and the inflection point of second-derivative plots are not constant. Results obtained for the samples were not reproducible. Even at high concentrations of 4-GBA (0.1 mol\L) only the wide jump of pH was achieved which significantly complicates the determination of titration end-point.

Non-aqueous titration of 4-GBA

Since aqueous titration gave unsatisfactory result, we developed another strategy for quantification of 4-GBA. Non-aqueous titration is used to quantify weak acids and bases [31] and is widely used for the evaluation of active substances in pharmaceutical substances formulations [22]. Salts of organic acids are typically titrated in solvents which have both protophilic and protogenic properties [32]. Considering this, we chose acetic acid as titrant, because it is non-toxic, cheap, environmentally friendly and readily available [33]. Also, 4-GBA dissolves completely in acetic acid in studied concentrations at room temperature (~ 25 °C) while it is not protonated upon dissolution. The obtained titrimetric curves for non-aqueous titration of 4-GBA are shown in Figure 3.

Non-aqueous titration conditions demonstrate excellent results in comparison to aqueous titration. The non-aqueous titration curve (Figure 3a) has a pronounced jump of potential, which allows uniquely determining

the end-point using acid-base indicators. In addition, excellent results were obtained when determining the equivalence point by the differential potentiometric method (Figure 3b). The increased basicity of 4-GBA is explained by the formation of acetonium ion ($CH_3COOH_2^+$) which results from interaction of perchloric acid and glacial acetic acid. The acetonium ion easily gives up proton to neutralize 4-GBA [33].

Validation

Thus, a conclusion can be made that non-aqueous titration using glacial acetic acid as a solvent and perchloric acid as titrant meets the main requirements for the quantitative determination of 4-GBA in laboratory and industrial conditions: it is express, easily accessible and precise. According to the requirements of world pharmaceutical standards, any quantification procedure must pass the validation process. Titrimetric methods were validated according to the procedures described in ICH guidelines Q2 (R1) for validation of analytical methods [34]. Validation was carried out according to the following indicators: specificity, linearity, accuracy, recovery, intermediate precision.

Specificity

The specificity of the method was examined by the absence of response on the related impurities that can influence the titration result. Methyl ether of 4-GBA (ME-4-GBA) was chosen as a related impurity because it is an intermediate compound in the synthesis of 4-GBA [24]. 4-GBA and its mixtures with ME-4-GBA (25, 50, 75 and 100% of the 4-GBA aliquot) were titrated (Table 1, entries 1-5 for Method A and entries 9-13 for Method B). For identification of possible excipients interference on quantification, 4-GBA was titrated in presence of several typical excipients for oral and injectable formulations of the following composition: talc (50% w/w), calcium gluconate (20% w/w), lactose (28.4% w/w), magnesium stearate (0.5% w/w), kollidon (0.1% w/w), aerosol (1% w/w). The results are shown in Table 1 (entries 6-8 for Method A and entries 14-116 for Method B).

As can be seen from Table 1, the addition of ME-4-GBA up to 100% of 4-GBA aliquot does not affect the result. The presence of excipients does not affect the quantitative determination as well. The latter indicates non-aqueous titration of 4-GBA in acetic acid as excellent method for its quantification in pharmaceutical preparations. Thus, we can conclude that both methods A and B are specific for quantitative determination of 4-GBA.

Linearity

Linearity was studied in the range 80-120% of the test concentration [34]. LOD and LOQ value of method A were detected by signal-to-noise ratio (S/N), which was 3 for LOD and 10 for LOQ. LOD and LOQ for method B was not calculated, because at low concentrations of 4-GBA determination of end-point (color change) accurately is impossible. Obtained data is show in Table 2, both methods demonstrate good linearity with excellent correlation coefficient.

Precision

Precision was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by the analysis of three samples during the same day, and the intermediate precision was studied by comparing the analyses on three different days. The analyses were carried out in triplicate and results were expressed as the relative standard deviation (RSD) of the analytical measurements. The results are shown in Table 3. As can be seen from Table 3, both methods A and B are precise because of RSD is more than 2%.

Accuracy

Accuracy was determined by recovery of 80%, 100%, 120% of the test concentration of 4-GBA from a standard solution [34]. The obtained percentages of the drug recovered, and standard deviations of the analysis are shown in Table 4. The percentage range recovery of 4-GBA was from 98.8 to 101.1% and from 98.1 to 103.2% for method A and method B, respectively. These ranges are suitable for the quantitative analysis of pharmaceutical substances.

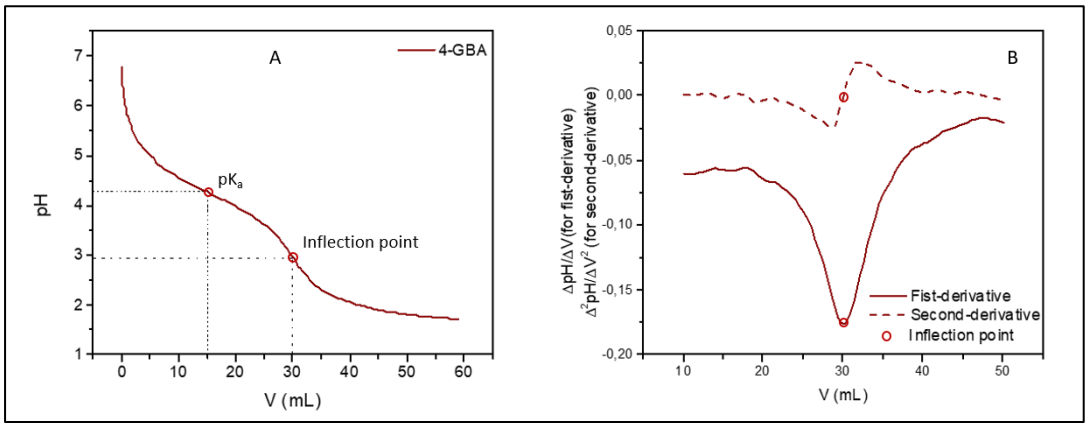

Figure 2. Graphical representation of aqueous titration analysis of 4-GBA with 0.1 N hydrochloric acid as titrant (a) and first (solid line), second (dashed line) derivative plot (b).

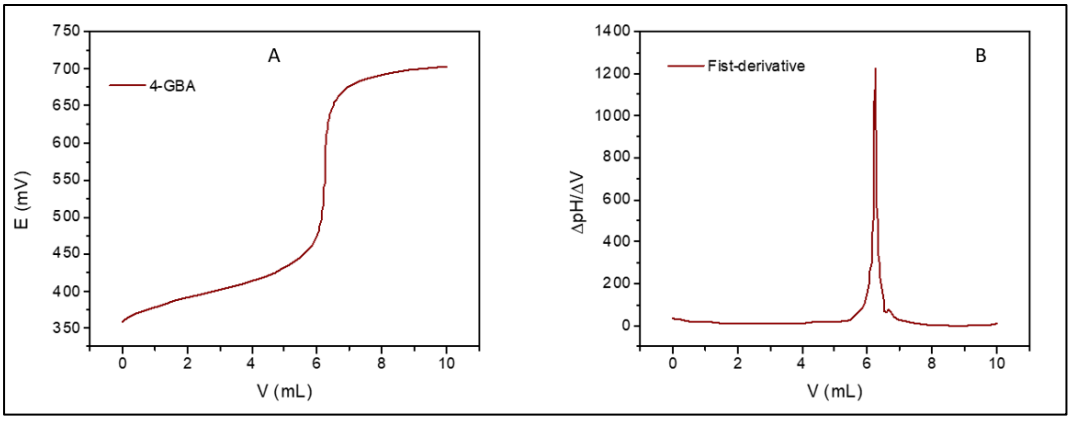

Figure 3. Non-aqueous titration curves of 4-GBA in (a) zero and (a) first order

Table 1. Results of specificity determination

Entry	Method	4-GBA (mg)	ME-4-GBA (mg)	Mix* (mg)	V (mL)	Assay (%)	Mean Assay
1	A	201.5	0.0	0	6.275	99.7	100.08 ± 0.59
2		198.3	50.3	0	6.150	100.1	
3		200.1	103.2	0	6.225	99.8	
4		196.3	154.8	0	6.025	101.1	
5		204.8	201.3	0	6.375	99.7	
6		200.4	0	100	6.335	101.79	100.12 ± 1.18
7		198.4	0	200	6.125	99.41	
8		203.1	0	300	6.255	99.17	

Table 1 (cont'd). Results of specificity determination

Entry	Method	4-GBA (mg)	ME-4-GBA (mg)	Mix* (mg)	V (mL)	Assay (%)	Mean Assay
9	B	200.3	0.0	0	6.2	98.7	100.56 ± 1.43
10		199.6	49.7	0	6.1	101.6	
11		201.7	101.1	0	6.3	99.4	
12		200.4	150.5	0	6.2	102.0	
13		198.8	198.7	0	6.2	101.1	
14		199.4	0	100	6.3	101.7	100.6 ± 1.4
15		200.7	0	200	6.3	101.1	
16		201.9	0	300	6.2	98.9	

* Mixture of excipients

Table 2. Results of linearity determination

Parameter	Results	
	Method A	Method B
Regression equation	y = 0.0312x - 0.005	y = 0.0331x - 0.37
Slope	0.0312	0.0331
Intercept	0.005	0.37
Correlation coefficient (r^2)	0.9995	0.9947
LOD	5.4 µg/mL	N/A*
LOQ	7.5 µg / mL	N/A*

*not applicable

Table 3. Precision of titration experiments

Method	Weight (mg)	Intra-day accuracy and precision (n=3)			Inter-day accuracy and precision (n=3)		
		Measured (mg)	RE (%)	RSD (%)	Measured (mg)	RE (%)	RSD (%)
A	181.4	180.9	0.26	0.61	182.5	0.59	1.56
	200.3	200.9	0.28	0.53	201.0	0.37	1.09
	219.8	218.9	0.39	0.46	219.5	0,15	0.88
B	180.3	181.7	0.79	1.52	181.8	0.81	1.99
	200.1	201.8	0.83	1.28	201.7	0.78	1.55
	220.3	221.3	0.45	1.23	219.8	0.21	1.63

Table 4. Results of accuracy and recovery

Method	Weight (mg)	Measured (mg)	Recovery (%)	Recovery (mean) (%)	RSD (%)
A	160.3	160.14	99.9	100.0	0.21
	160.8	161.12	100.2		
	161.1	160.78	99.8		
	200.4	198.00	98.8	99.8	1.2
	200.9	203.11	101.1		
	201.3	200.09	99.4		
	241.2	243.13	100.8	99.6	1.0
	240.8	238.15	98.9		
	240.3	238.14	99.1		
B	160.1	163.20	98.1	99.2	1.11
	161.7	162.84	99.3		
	160.8	160.32	100.3		
	200.1	202.74	98.7	101.4	1.79
	201.4	197.06	102.2		
	200.8	194.57	101.2		
	241.3	237.73	101.5	101.3	1.16
	240.2	240.92	99.7		
	239.8	235.33	101.9		

Conclusion

Fast, simple and reliable titrimetric method for quantitative determination of 4-GBA was developed and validated. This method allows quantitative determination 4-GBA in each batch of the substance in subsequent preclinical studies without use of standard samples. The aqueous titrimetric assay was unsuccessful and demonstrated insufficient accuracy due to the wide jump of pH. Instead, non-aqueous titrimetric method using glacial acetic acid and perchloric acid was applied to the determination of pure authentic samples and some of their pharmaceutical preparations. The non-aqueous titration demonstrated rather well shaped end-points with high potential jumps both in potentiometric and visual methods. Statistical tests indicate that the proposed titrimetric method appear to be equally suitable for routine determination of 4-GBA in pharmaceutical formulation, bulk and in pure form.

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