

# Instrumental analysis of chloroquine and hydroxychloroquine in different matrices

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## ABSTRACT

Currently, the emergence of a novel human corona virus, COVID- 19, has become a global burden causing severe symptoms and death. In this literature review, we will introduce most of up-to- date reported methods

that have been developed for determination of two important antimalarial and anti-COVID-19 drugs which are Chloroquine and Hydroxychloroquine in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples.

**Key Words:** COVID-19; Chloroquine; Hydroxychloroquine; degradation products; biological samples.

## INTRODUCTION

Quinoline derivatives such as Chloroquine (CQ) and Hydroxychloroquine (HCQ) are used to treat malaria. These drugs have not yet been licensed for the treatment of viral infections, and there are no well-controlled, prospective, randomized clinical trials or evidence to support their use in patients with coronavirus disease (COVID-19) [1].

Due to the rapidly spreading pandemic caused by extreme acute respiratory syndrome-coronavirus-2, dubbed coronavirus disease (COVID-19) in 2019, CQ and HCQ are now gaining worldwide attention in treatment strategy. The use of CQ/HCQ in the treatment of COVID-19 clinical syndrome has been restricted thus far [2].

Due to the current importance of these drugs in the treatment of pandemic COVID-19, this literature focuses on different analytical methods that have been developed for the determination of these drugs in different pharmaceutical and biological samples.

## ANALYTICAL METHODS

Various techniques were used for the analysis of CQ and HCQ in pure forms, in their pharmaceutical formulations and biological fluids [Table 1-5]. The available reported methods in the literature can be summarized as follows:

**TABLE 1**

### Spectroscopic methods

| Drugs | Matrix | Method or Reagents | $\lambda_{max}$ (nm) | Linearity | LOD | Ref |
|-------|--------|--------------------|----------------------|-----------|-----|-----|
|-------|--------|--------------------|----------------------|-----------|-----|-----|

|     |                                           |                                                 |     |        |       |     |
|-----|-------------------------------------------|-------------------------------------------------|-----|--------|-------|-----|
| HCQ | Pharmaceutical formulation                | UV Spectrophotometry using 0.1N HCl as solvent  | 343 | 10     | ----- | [3] |
| HCQ | Pharmaceutical formulation                | UV Spectrophotometry using 0.01M HCl as solvent | 342 | 08-Dec | 0,38  | [4] |
| HCQ | Raw material                              | UV Spectrophotometry using dil. HCl as solvent  | 343 | 10     | ----- | [5] |
| HCQ | Tablets                                   | UV Spectrophotometry (AUC method)               | 251 | 02-Dec | 0,37  | [6] |
| CQ  | Tablets                                   | UV Spectrophotometry                            | 343 | 30,56  | 0,99  | [7] |
|     | Tablets, injections and syrup formulation | Frist derivative spectrophotometry              | ns. | 2,5-50 |       |     |

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|                                       |                                      |                                                                               |           |                               |                        |      |
|---------------------------------------|--------------------------------------|-------------------------------------------------------------------------------|-----------|-------------------------------|------------------------|------|
| CQ                                    |                                      |                                                                               | 349       | µg/mL                         | -----                  | [8]  |
| CQ,<br>Pyrimethamine and Trimethoprim | Pharmaceutical formulation and urine | Ion pair complex with bromocresol purple                                      | 420       | 1,25-8,75 µg/mL<br>Up to 120  | 0,125 µg/mL            | [9]  |
| CQ and its metabolite                 | Urine samples                        | Ion pair complex with bromothymol blue                                        | 410       | mg/L                          | 3 mg/L                 | [10] |
| CQ and Pyrimethamine                  | Tablets                              | Ion-pair complex with molybdenum and thiocyanate                              | 467       | Feb-42 µg/mL                  | -----                  | [11] |
| CQ                                    | Urine samples                        | Ion-pair complex with methyl orange                                           | 420       | Up to 32 µg/mL                | 0,3 µg/mL              | [12] |
| CQ                                    | Urine samples                        | Colorimetric test, Dill-Glazko's test, and UV/Visible absorbance spectrometry | 329 & 343 | -----                         | 15 mg/L and 0,13 µg/mL | [13] |
| CQ and its degradation products       | Pharmaceutical formulation           | UV Spectrophotometry                                                          | 220       | 01-Oct µg/mL                  | 0,27 µg/mL             | [14] |
| CQ                                    | Tablets, suspension and injections   | Ion pair complex with bromocresol green and bromocresol purple                | 420       | Jan-20 µg/mL<br>-0,5-12 µg/mL | 0,15 µg/mL             | [15] |
| CQ                                    | Tablets                              | UV Spectrophotometry using 0.06 M monosodium phosphate buffer pH 6.8          | 343       | 7,2-19,2 µg/mL                | -----                  | [16] |
| CQ                                    | Tablets                              | Difference spectrophotometry                                                  | 285 & 345 | 50-250 µg/mL                  | -----                  | [17] |
| CO                                    | Tablets                              | UV spectrometry                                                               | 254       | 10-64 µg/mL                   | -----                  | [18] |

TABLE 2

## Spectrofluorimetric methods

| Drugs | Matrix                          | Fluorogenic Reagent (Method)          | $\lambda_{ex}$ (nm) | $\lambda_{em}$ (nm) | Linearity Range   | LOD                   | Ref. |
|-------|---------------------------------|---------------------------------------|---------------------|---------------------|-------------------|-----------------------|------|
| CQ    | Tablets                         | 0.05 M H <sub>2</sub> SO <sub>4</sub> | 339                 | 368                 | 01-Oct µg/mL      | 0.77 µg/mL<br>3.3×1   | [19] |
| CQ    | Tablets and spiked human plasma | M 4.0 sodium dodecyl sulfate (pH 10)  |                     |                     | 5-0.003 µg/mL     | 0- <sup>3</sup> µg/mL | [20] |
| CQ    | suspension                      | 10 mg/ml in distilled water           | 330                 | 369<br>330–400      | Up to 0.625 mg/mL | -----                 | [21] |

TABLE 3

## Chromatographic methods

| Drugs                           | Matrix          | Column                                | Mobile phase                                                            | Detector                         | Linearity       | LOD        | Ref  |
|---------------------------------|-----------------|---------------------------------------|-------------------------------------------------------------------------|----------------------------------|-----------------|------------|------|
| HCQ and its metabolites         | Human plasma    | Poly (styrene divinylbenzene) packing | Methanol (80): Water (20) with triethylamine, (pH 11)                   | Fluorescence detection at 337 nm | -4              | 0.1 µg/mL  | [22] |
| HCQ and its major metabolites   | Plasma          | CN (cyano-bonded silica)              | (79:20:1) 0.03 M sodium phosphate buffer, pH 7.0:ethanol : acetonitrile | UV at 320 nm.                    | 144 ng/mL       | 14 ng/mL   | [23] |
| HCQ and three major metabolites | Blood and urine | ODS                                   | Hexane: tert butyl ether: 0.5% n-butylamine in methanol (1:1:1)         | Fluorescence detection at 380 nm | Upto 1000 ng/mL | 5-10 ng/mL | [24] |

Instrumental analysis of chloroquine

|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
|------------------------------------------------------|------------------------|------------------------------------------------------------|------------------------------------------------------------------|---------------------------------------------|--------|-----|-----------|------|----------------------------------|-------------------------|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|----------------------------------|------------------|---------|------|
| HCQ and three major metabolites                      | Plasma                 | ODS (cyano column - chiral-AGP column)                     | Phosphate buffer-methanol: Triethanolamine (78:22:1:0.0)         | Fluorescence detection at 385 nm            | –2     | 24  | 1 < ng/mL | [25] | HCQ                              | Blood                   | column                                             | and methanol acidified with 0.1% formic acid                                                                                  | MS/MS                            | 2444 ng/mL       | [29]    |      |
|                                                      |                        |                                                            | 8, v/v)                                                          |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
| quinine, HCQ, CQ, and desethyl chloroquine           | Serum, and whole blood | CN (sulfophenylpropyl-modified silica)                     | 0.015M                                                           | nm)                                         |        | 4.4 |           | [26] | HCQ, minocycline and doxycycline | Blood                   | Acquity BEH Phenyl (2.1 × 50 mm 1.7 μm)            | 1% Triethylamine and 1 mM oxalic acid in water adjusted to pH 2.4 with orthophosphoric acid at 85%                            | UV at 343 nm                     | 0.1 - 20.0 μg/mL | 4.2     | [30] |
|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
| HCQ, CQ and some corticosteroids                     | Serum                  | Nova-Pak C <sub>18</sub> (150 x 3.9 mm, 0 μm)              | K <sub>2</sub> HPO <sub>4</sub> buffer: Methanol: ACN (20:30:50) | Fluorimetry (excitation wavelength, 215 nm) | 24-Feb | 40  |           | [26] | HCQ                              | Blood                   | Hypersil C18 column (250×6 mm, 5 μm)               | Water and (acetonitrile: methanol: 50:50, v/v) in 75:25 v/v ratio, with sodium 1-pentane sulfonate and phosphoric acid (pH 3) | UV at 343 nm                     | 0.1 - 20.0 μg/mL | 4.2     | [30] |
|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
| Enantio-selective analysis of the metabolites of HCQ | Rat liver microsomes   | Chiralpak AD-RH column protected with an RP-8 guard column | 4.705 g NH <sub>4</sub> ClO <sub>4</sub>                         | UV at several wavelength                    | 25-    |     |           | [27] | HCQ and its metabolites          | Blood                   | octadecyl silane Luna C <sub>18</sub>              | A water–methanol–acetonitrile (02:14:02, v/v/v) mixture containing                                                            | Fluorescence detection at 370 nm | –1               | 1       | [31] |
|                                                      |                        |                                                            | Methanol: water (98.5:1.5)+                                      |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
| HCQ and its active metabolites                       | Hypersil Gold aQ       | A gradient of water                                        | hexane:isopropanol (92:8, v/v)                                   | UV at 343 nm                                |        |     |           | [28] | HCQ and its metabolites          | Mouse blood and tissues | Thermo Aquasil C <sub>18</sub> (50 × 4.6 mm, 2 μm) | A gradient elution using 0.2% formic acid and 0.1% formic acid in methanol                                                    | MS/MS                            | 2000 ng/mL       | 1 ng/mL | [33] |
|                                                      |                        |                                                            | plus 0.1% diethylamine                                           |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |
|                                                      |                        |                                                            |                                                                  |                                             |        |     |           |      |                                  |                         |                                                    |                                                                                                                               |                                  |                  |         |      |

|                         |             |                                                             |                                                                                                                                          |                                  |        |     |      |   |                                        |              |                                                                         |                                                                                                          |                                  |       |       |      |
|-------------------------|-------------|-------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|--------|-----|------|---|----------------------------------------|--------------|-------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|----------------------------------|-------|-------|------|
| HCQ                     | Human blood | Agilent ZORBA X Eclipse XDB – C <sub>8</sub>                | 0.10% formic acid–acetonitrile (94:6, v/v)                                                                                               | MS/MS                            | –0     | 2   | [34] | L | HCQ                                    | Tables       | X-terra phenyl column (204 × 0.6 mm, 5 µm)                              | Acetonitrile and buffer in the ratio 70:30 v/v respectively                                              | UV at 220 nm                     | –4.24 | 4.4   | [39] |
|                         |             |                                                             |                                                                                                                                          |                                  |        |     |      |   |                                        |              |                                                                         |                                                                                                          |                                  |       |       |      |
| HCQ                     | Tables      | Agilent Zorbax C <sub>8</sub>                               | 0.01M of 1-pentane sulfonic acid and 0.02% of Ortho phosphoric acid in purified water with acetonitrile and methanol (800: 100: 100 v/v) | UV at 254 nm                     | –20    | 244 | [35] | L | HCQ and its two metabolites            | Blood        | YMC-Triart C <sub>18</sub> column (250 × 4.6 mm, 0 µm)                  | mM 24 sodium phosphate buffer solution containing 0.25% triethylamine (pH 8.0)—acetonitrile (60:40, v/v) | Fluorescence detection at 405 nm | –2    | 10    | [41] |
|                         |             |                                                             |                                                                                                                                          |                                  |        |     |      |   |                                        |              |                                                                         |                                                                                                          |                                  |       |       |      |
| HCQ and its metabolites | Blood       | U-HPLC RP <sub>18</sub>                                     | Piperazine buffer (46.4 mM, pH = 9.8) and acetonitrile (68:32, v/v)                                                                      | Fluorescence detection at 390 nm | 125    | –   | [36] | L | CQ and HCQ                             | Human plasma | Agilent Zorbax SB-C <sub>18</sub>                                       | %4.1 %20 trifluoroacetic acid and 5% methanol, evolved in 8 minutes to 50% methanol                      | UV at 343 nm                     |       |       | [42] |
|                         |             |                                                             |                                                                                                                                          |                                  |        |     |      |   |                                        |              |                                                                         |                                                                                                          |                                  |       |       |      |
| HCQ enantiomers         | Blood       | Chiralpak AD-H column 4.6 mm × 100 mm, 5 µm))               | n-hexane-isopropanol (93:7, v/v) plus 0.5% DEA into hexane                                                                               | UV at 343 nm                     | Jan-25 | 4.4 | [37] | L | HCQ and its metabolites & Azithromycin | Human plasma | PFP column (2.0 × 50 mm, 3 µm)                                          | 0.1% TFA in acetonitrile                                                                                 | MS/MS                            | to 2  | 4.2   | [43] |
|                         |             |                                                             |                                                                                                                                          |                                  |        |     |      |   |                                        |              |                                                                         |                                                                                                          |                                  |       |       |      |
| HCQ and its metabolites | Blood       | XTerra phenyl® column (250 × 4.6 mm, 0 µm; Waters, MA, USA) | Glycine buffer/sodium chloride (pH 9.7, 100 mM) and methanol (46:54; v/v)                                                                | Fluorescence detection at 380 nm | –4     | 20  | [38] | L | HCQ                                    | Blood        | Kinetex C <sub>8</sub> (2.1 × 50 mm, 2.2 µm, Phenomenex, Torrance, USA) | 0.10% formic acid and 0.01% and 0.01% triethylamine in water (or acetonitrile)                           | MS/MS                            | 2000  | ng/mL | [44] |
|                         |             |                                                             |                                                                                                                                          |                                  |        |     |      |   |                                        |              |                                                                         |                                                                                                          |                                  |       |       |      |
| HCQ and its metabolites | Blood       |                                                             |                                                                                                                                          |                                  |        |     |      | L | HCQ and its                            | Human        |                                                                         | Hexane/methanol/ethanol (96:2:2, v/v/v) plus 0.2%                                                        | UV at 343                        | –04   |       |      |
|                         |             |                                                             |                                                                                                                                          |                                  |        |     |      |   |                                        |              |                                                                         |                                                                                                          |                                  |       |       |      |

|                                                          |                                      |                                                              |                                                                                                       |              |                 |           |      |
|----------------------------------------------------------|--------------------------------------|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|--------------|-----------------|-----------|------|
| major metabolites                                        | urine                                | Chiralcel OD-H                                               | diethylamine                                                                                          | nm           | 1444 ng/mL      |           | [45] |
| CQ and primaquine                                        | Tablets                              | Hypersil C18                                                 | Acetonitrile and 0.1% aqueous triethylamine                                                           | UV at 260 nm | 4.21 mg/mL      | 1 mg/mL   | [46] |
| CQ, mono-desethyl chloroquine, diazepam, and nordiazepam | Blood                                | Bondapak C <sub>8</sub> column (30 cm x 3.9 mm, 14 µm)       | 0.0125M potassium dihydrogen phosphate solution and 1N phosphoric acid (99.90:0.10 v/v): acetonitrile | UV at 343 nm | -4 ng/mL        | 14 ng/mL  | [47] |
| CQ and desethyl chloroquine                              | Blood                                | Thermo Hypersil Gold C <sub>18</sub> , 250 mm x 2.1 mm, 5 µm | 1% diethylamine, acetonitrile and methanol (20:55:25, v:v:v)                                          | UV at 256 nm | 25 - 1500 ng/mL | 0.2 ng/mL | [48] |
| CQ and quinine                                           | Human plasma, erythrocytes and urine | Inertsil silica (250 mm x 4 mm, 0 µm)                        | ammonia solution (92.7:7.5, v/v)                                                                      | nm           | -20 1444 ng/mL  | 0 ng/mL   | [49] |
| CQ, Pyrimethamine and Cetirizine                         | Human serum                          | Purosphere C <sub>18</sub>                                   | methanol: water (70:30) at pH of 2.8                                                                  | UV at 230 nm | 144-1 µg/mL     |           |      |

TABLE 4

## HPLC methods

| Drug                             | Matrix                   | Mobile phase                                                               | Stationary phase                                  | Detection                  | Linearity range | LOD       | Ref  |
|----------------------------------|--------------------------|----------------------------------------------------------------------------|---------------------------------------------------|----------------------------|-----------------|-----------|------|
| HCQ,                             |                          |                                                                            |                                                   |                            | 120             | 46        |      |
| Methotrexate, and Sulfasalazine. | Serum and urine          | Ethyl acetate : methanol 25% : ammonia (8:2:3 v/v)                         | Silica gel 60 F 254 plates                        | UV at 340 nm               | - 100 4 ng/mL   | 84 ng/mL  | [50] |
| CQ and primaquine                | Tablets and capsules     | Hexane: diethyl ether: methanol: diethyl aniline (37.5 :37.5:25:0.5 v/v ). | Silica gel 60 F 254 plates                        | UV at 254 nm.              | 4- 33 µg/mL     | 1.6 µg/mL | [51] |
| CQ and Desethyl chloroquine.     | Plasma and urine sample. | Toluene : diethyl aniline (9:1 v/v ).                                      | Silica gel plates                                 | Fluorescence               | -----           | 1 µmol/L  | [52] |
| CQ and desethyl chloroquine      | Urine                    | Ammonia : methanol in methyl tert butyl ether (MTBE) 18:8 v/v .            | Silica gel 60 plates with impregnated fluorophore | Colorimetric and UV 254 nm | 01-May µg/mL    | 0.2 µg/mL | [53] |
| CQ ,                             |                          |                                                                            |                                                   |                            |                 |           |      |
| Azethro mycin and paraceta       | Tablets                  | Methanol 25%:amonia (100 : 1.5 v/v) .                                      | Silica gel 60 F plates                            | UV at 254nm                | 0.1- 10 mg/mL   | ---       | [54] |

TABLE 5

## Electrochemical determination

| Drugs | Matrix | Electrode                            | Linearity | LOD | Ref. |
|-------|--------|--------------------------------------|-----------|-----|------|
|       |        | Cathodically pretreated (CPT) boron- |           |     |      |

|     |                               |                                                                                                                                                                             |                                                   |                            |      |     |                            |                                                                                                |                                                   |                            |      |
|-----|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|----------------------------|------|-----|----------------------------|------------------------------------------------------------------------------------------------|---------------------------------------------------|----------------------------|------|
| HCQ | Tablets and urine             | doped diamond (BDD) electrode                                                                                                                                               | $\mu\text{M}$ 1.2 - 4.1                           | 0.06 $\mu\text{M}$ mol/L   | [55] | CQ  | Tablets and                | (PMO) and multi-walled carbon nanotubes (MWCNT)                                                | $1.0 \times 10^{-7}$ - $3.5 \times 10^{-6}$ mol/L | $8.9 \times 10^{-8}$ mol/L | [64] |
|     |                               | Polymeric membrane (PMEs) and modified carbon paste (CPEs) electrodes which are based on sodium tetraphenyl borate (NaTPB) or ammonium reineckate (Rt) as sensing materials |                                                   |                            |      |     |                            | covalent modification of glassy carbon electrode (GCE) by self-assembly of a novel Schiff base |                                                   |                            |      |
| HCQ | In the presence of Uric Acid  |                                                                                                                                                                             |                                                   |                            | [64] | HCQ |                            |                                                                                                | $2 \times 10^{-5}$ to $5 \times 10^{-4}$ M        | 11.2 $\mu\text{g/mL}$      | [64] |
|     |                               |                                                                                                                                                                             |                                                   |                            |      |     |                            |                                                                                                |                                                   |                            |      |
| HCQ | Tablets and urine             |                                                                                                                                                                             | $1.0 \times 10^{-2}$ - $1.0 \times 10^{-6}$ mol/L | $5.0 \times 10^{-2}$ mol/L | [56] |     |                            |                                                                                                |                                                   |                            |      |
| HCQ | Tablets                       | Glassy carbon electrode                                                                                                                                                     | $2 \times 10^{-5}$ - $5 \times 10^{-4}$ mol/L     | 11.2 $\mu\text{g/mL}$      | [57] |     |                            |                                                                                                |                                                   |                            |      |
| CQ  | Tablets and human serum       | Glassy carbon electrode modified with reduced graphene oxide on WS <sub>2</sub> quantum dots                                                                                | 0.5 - 0.02 $\mu\text{M}$                          | 4.10 $\mu\text{M}$         | [58] | CQ  | Pharmaceutical formulation | Boron-doped diamond (BDD) electrode                                                            | 0.01 - 0.25 $\mu\text{mol/L}$                     | 2 nmol <sub>1</sub>        | [59] |
|     |                               |                                                                                                                                                                             |                                                   |                            |      |     |                            |                                                                                                |                                                   |                            |      |
| CQ  | Tablets and biological fluids | Poly(vinyl chloride) membrane electrodes Carbon paste electrode modified with Cu(OH) <sub>2</sub> nano-wire                                                                 | 0.01 - 100 mM                                     | 0.02 mM                    | [60] |     |                            |                                                                                                |                                                   |                            |      |
| CQ  | Tablets                       |                                                                                                                                                                             | 0.068 - 6.88 $\mu\text{g/mL}$                     | 0.01 $\mu\text{g/mL}$      | [61] |     |                            |                                                                                                |                                                   |                            |      |
| CQ  | Pharmaceutical Preparations   | Two types of polyvinyl chloride (PVC) carbon paste and dsDNA-modified carbon paste electrodes                                                                               | $1.0 \times 10^{-1}$ - $1.0 \times 10^{-6}$ M     | $3.2 \times 10^{-6}$ M     | [62] | CQ  |                            |                                                                                                |                                                   |                            | [63] |
| CQ  | Serum                         | glassy carbon electrode modified with electrochemically polymerised methyl orange                                                                                           | $1.0 \times 10^{-2}$ - $1.0 \times 10^{-0}$ mol/L | $3.0 \times 10^{-0}$ mol/L |      |     |                            |                                                                                                |                                                   |                            |      |

## CONCLUSION

This literature review represents an up to date survey about all reported methods that have been developed for the determination of Chloroquine and Hydroxychloroquine in their pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as spectrophotometry, Spectrofluorimetry, liquid chromatography, electrochemistry, etc

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