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Regulatory Perspective Reverse Engineering Analysis of the Mast Cell Stabilizer and the Histamine Receptor Antagonist (Olopatadine HCl): Instrumental and Classical Methods for Multiple Formulations

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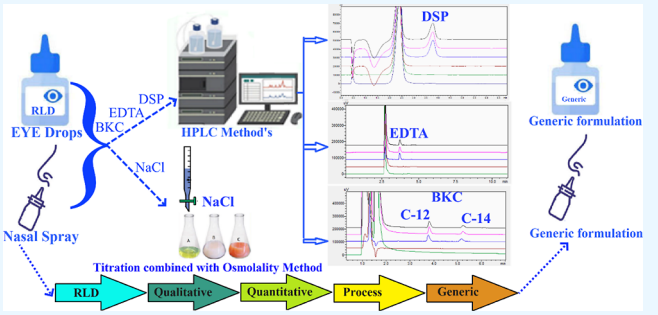
ABSTRACT: This study evaluates the unknown qualitative (Q1) and quantitative (Q2) formulas for nasal spray and ophthalmic solution formulations of olopatadine HCl by classical and instrumental techniques to match the generic formula with reference-listed drugs to avoid clinical study. Reverse engineering of olopatadine HCl nasal spray 0.6% and ophthalmic solution 0.1, 0.2% formulations was accurately quantified using a simple and sensitive reversed-phase high-performance liquid chromatography (HPLC) method. Both formulations possess similar components, namely ethylenediaminetetraacetic acid (EDTA), benzalkonium chloride (BKC), sodium chloride (NaCl), and dibasic sodium phosphate (DSP). These components were qualitatively and quantitatively determined using the HPLC, osmometry, and titration techniques. With derivatization techniques, EDTA, BKC, and DSP were determined by ion-interaction chromatography. NaCl in the formulation was quantified by measuring the osmolality and using the subtraction method. A titration method was also used. All the employed methods were linear, accurate, precise, and specific. The correlation coefficient was >0.999 for all components in all the methods. The recovery results ranged from 99.1 to 99.7% for EDTA, 99.1−99.4% for BKC, 99.8−100.8% for DSP, and 99.7−100.1% for NaCl. The obtained % relative standard deviation for precision was 0.9% for EDTA, 0.6% for BKC, 0.9% for DSP, and 1.34% for NaCl. The specificity of the methods in the presence of other components, diluent, and the mobile phase was confirmed, and the analytes were specific.



1. INTRODUCTION

Reverse engineering aims to produce a generic formulation that is qualitatively and quantitatively similar to the reference listed drug (RLD). In the reverse engineering process, the idea is to generate a formulation and recreate the exact formulation to be able to achieve all the properties associated with the actual formulation. The reverse engineering technique is also known as the deformulation technique. Parenteral drug product injections, topical products, ophthalmics, and otic solutions are eligible for a waiver of bio-equivalence (BE) studies by proving the formula's sameness with RLD products. Generic formulation submission to the Abbreviated New Drug Application (ANDA) is mandatory to show the physicochemical data matches the RLD product. Moreover, Q1 and Q2 should be identical for parenteral drug products. For the generic formulation to agree with RLD, it is necessary to do a deformulation study on RLD to know the undisclosed contents of the excipients.

Olopatadine HCl (OPT) has a dual therapeutic action and works as a mast cell stabilizer and histamine H1 receptor antagonist. OPT is available in multiple formulations, such as ophthalmic solutions, nasal sprays, and tablets. Molecular formulae and molecular weights are $C_{21}H_{24}ClNO_3$ and 373.9. In 2008, the ophthalmic formulation market reached 14 billion dollars in the United States of America (USA). As per IMS Health data, the overall market for OPT is nearly 230 million dollars. There is no generic product available for nasal sprays, and the 0.2% ophthalmic formulation and patents will expire in 2022 and 2023, respectively. The best time to develop the generic formulation is to get exclusivity for 6 months for the first filling. The current procedure will help make the generic formulation. Ahmed et al.[1] did the reverse engineering study for OPT nasal spray 0.1% only by utilizing the different solubility techniques, but the proposed article clearly stated that the research was done at the academic level. The methods used for the study had a sensitivity issue. Using these methods

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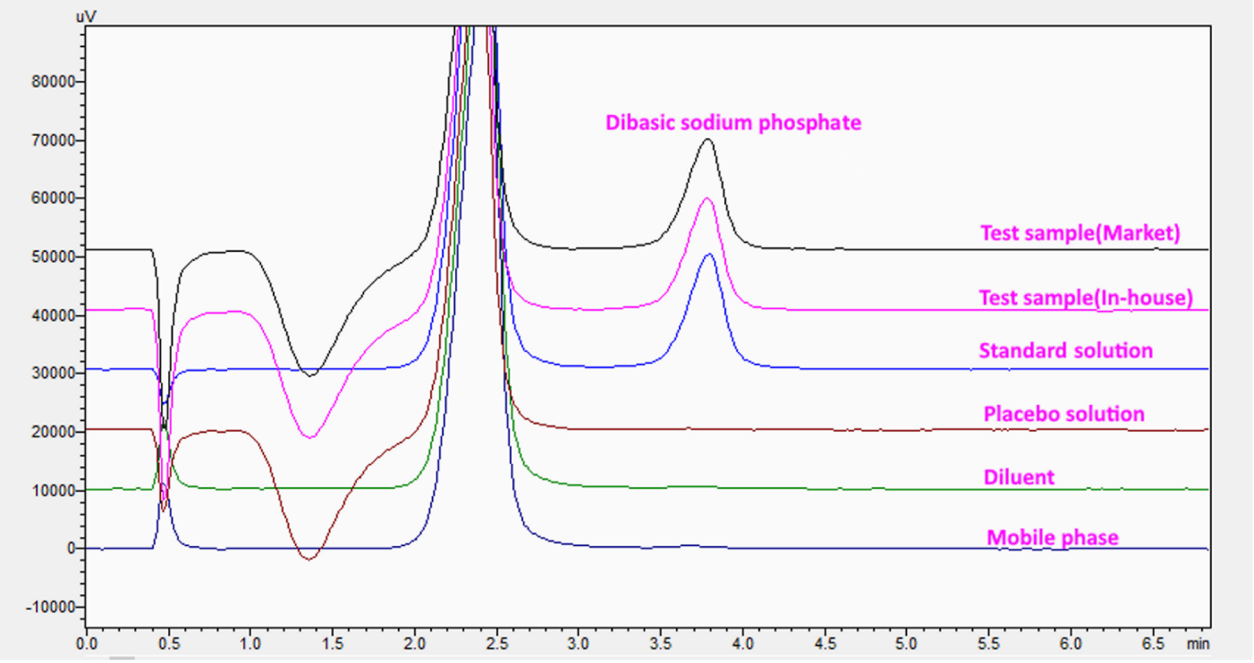

Figure 1. Final chromatogram with the following optimized conditions: a flow rate of 1.0 mL/min, a column temperature of 25 °C, an injection volume of 50 μL, and a wavelength of 288 nm with a waters IC-Pak anion (50 × 4.6 mm, 10 μm) column.

from the industrial point of view may raise queries, and justifying Q2 is a huge challenge for regulatory bodies.[2−6]

The current research targetes reverse engineering of both ophthalmic and nasal spray formulations. The study on marketed RLD aims to make a generic formulation to match the physicochemical properties. Ophthalmic solution 0.1 and 0.2% and nasal spray 0.6% formulations contain the following excipients: ethylenediaminetetraacetic acid (EDTA) as a chelating agent, benzalkonium chloride (BKC) as a preservative, dibasic sodium phosphate (DSP) as a buffer system, and NaCl as a tonicity agent. Sodium hydroxide and hydrochloric acid were used for pH adjustment. These quantities do not impact the formulation and are not required to match Q1 and Q2 with RLD.

EDTA used as a chelating agent in the formulation is a very low UV index compound, typically quantified using high-performance liquid chromatography (HPLC) with a UV detector. Our study used a derivatization process with an ion-interaction chromatography technique to match Q1 and Q2 against RLD. Although a few reports are available for EDTA analysis,[7−11] the reported method has an advantage in run time and derivatization process over the literature methods.

BKC is alkyldimethylbenzylammonium chloride, a quaternary ammonium salt. BKC is determined using HPLC and matching Q1 and Q2 with RLD. Few BKC quantification articles are published,[12−14] but the reported method has the advantage of specificity for this molecule and a shorter run time.

DSP is not UV active and is determined using HPLC and derivatization. The ion-interaction chromatography technique was also used. Most of the reported methods for determining DSP used titration and high-end techniques with HPLC.[15−18] The current approach is more straightforward and accurate.

NaCl is determined by Mohr's titration and the osmometer subtraction method to revalidate the results. Relative to the reported NaCl determination techniques,[19−22] the current approach has the advantages of analysis time, sensitivity, and specificity for this molecule.

The main challenges in method development are the determination of EDTA, a low UV index complex, and DSP, a UV inactive compound, in the presence of excipients and API using the most reliable and sensitive chromatographic technique. BKC, the UV-active species, was obtained by a simple HPLC method in the presence of excipients and API. The determination of NaCl by titration is challenging to quantify accurately. Developing a protocol suitable for a small-scale laboratory, specific with placebo, and interference-free with other components is demanding. The proposed method is cost-effective, analyst-friendly, and reliable for making a generic formulation that matches the RLD and avoids a BE study meeting all the requirements. All the components chemical structures are shown in Figure S1.

2. RESULTS AND DISCUSSION

2.1. Chromatography Optimization for DSP. DSP does not have a chromophore; hence, it is difficult to quantify by HPLC. Based on the literature, the initial chromatography conditions were initiated with an IC-Pak (50 × 4.6 mm, 10 μm) column. The column material contains polymethacrylate resin with a quaternary ammonium functional group. It interacts with the anion (HPO_4^{2-}) and adsorbs the anion in the stationary phase. The mobile phase selection was also critical, as DSP contains a cation (Na^+) and an anion (HPO_4^{2-}). Regular phosphate buffers are not suitable due to interference with DSP. The literature indicates that the mobile phase should be an aqueous solution with a suitable ion-interaction reagent.

Potassium hydrogen phthalate (PHT) was added to the mobile phase as an ion-interaction reagent to obtain high background absorption in the mobile phase. It will increase the lipophilicity, which will lead to retaining the anion in the reverse phase. Different buffers, such as 0.1 mM nitric acid

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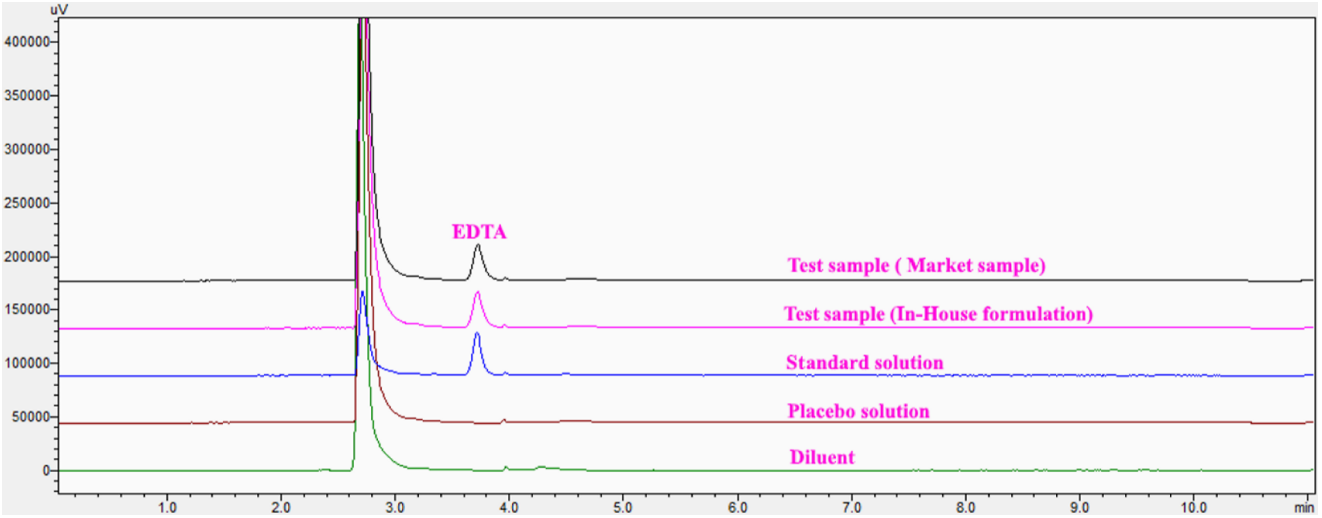

Figure 2. Final chromatogram with the following optimized conditions: a flow rate of 1.0 mL/min, a column temperature of 30 °C, an injection volume of 50 μL, and a wavelength of 260 nm with the Phenomenex Ultracarb C8 (150 × 4.6 mm, 5 μm) column.

buffer, 1.3 mM boric acid buffer, and 1.3 mM gluconic acid buffer, were selected and prepared for the mobile phase by adding PHT. A 50 μL of the DSP sample solution prepared in water was injected into HPLC with a 1.0 mL/min flow rate. No peaks were observed in the gluconic acid and boric acid buffers. The phosphate anion was eluted in the nitric acid buffer, and the peak was eluted with a distorted peak shape. The nitric acid buffer strength was increased from 0.1 to 100 mM to improve the peak shape. To optimize PHT concentrations in the mobile phase, different PHT concentrations were examined. DSP peak met the system suitability criteria with a 1.0 mM PHT concentration, which was considered for further analysis. At 288 nm, the monitoring wavelength, no interference was observed with diluent or placebo samples. Figure 1 illustrates the final optimized and interference-free chromatogram.

2.2. Chromatography Optimization for EDTA. EDTA has a low UV index; due to this reason, it is challenging to quantify by HPLC with the most commonly used UV detector. However, EDTA has a strong metal ion bonding nature that helps form a complex with metal ions. Copper (II) acetate solution was added to create an EDTA complex. The obtained complex improves the UV index and activates the chromophore of EDTA. This activation helped quantify the EDTA by HPLC. The chemical reaction of EDTA with copper (II) acetate formed the anionic complex, as shown below.

$$Cu^{2+} + (EDTA)^{2} \rightarrow Cu(EDTA)^{2-} + 2H^{+}$$

Optimization of chromatographic conditions was initiated with 10 mM sodium acetate pH 4.5 buffer with ACN in the ratio of (50:50 v/v) as the mobile phase and an Ultracarb C8 (150 × 4.6 mm, 5 μm) column with a flow rate of 1.0 mL/min. After Injecting 50 μL of EDTA standard solution, EDTA was not eluted. According to literature data, retention of EDTA on a reversed-phase stationary phase may be possible by adding the lipophilic cation to the mobile phase. The various lipophilic cations were added to the pH 4.5 sodium acetate buffer: cetyltrimethylammonium, tetrabutylammonium hydroxide (TBAH), and tetramethylammonium. The EDTA standard was prepared, and HPLC injected lipophilic cations with 10% TBAH into the aqueous mobile phase consisting of 30% ACN and gave positive results. Prepared the pH 4.5 buffer by adding TBAH, and prepare the mobile phase with acetonitrile in the (90:10 v/v) injected EDTA solution. The EDTA peak was eluted at about 12 min, the UV spectra were recorded, the maximum absorbance was identified at wavelength 260 nm, and it was considered for further trials. We conducted a series of experiments with different volumes of 10% TBAH, such as 15, 10, 8, and 5 mL, into the buffer solution to reduce the elution time of the EDTA complex. TBAH concentration played a key role in EDTA complex elution. The optimal volume of TBAH is 8 mL for 1000 mL of pH 4.5 buffer. The selected column temperature of 30 °C for the current study is based on the peak elution. The optimization of copper (II) acetate concentration in diluent was evaluated by preparing the diluent with different concentrations. The studied concentrations were 1, 5, 10, 15, and 20 mg/mL of copper acetate (II). These standard solutions were verified for suitability, and it was found that 10 mg/mL is suitable for the current study with an appropriate response when compared to other concentrations. The final optimized and interference-free chromatogram is shown in Figure 2.

2.3. Chromatography Optimization for BKC. BKC is a cationic surfactant and contains C_{10}, C_{12}, C_{14}, and C_{16} homologs. The amount of C_{10} and C_{16} homologs are present in a small amount; C_{12} and C_{14} homologs are mainly present. The chromatographic method optimization was initiated with the Ultracarb C8 (150 × 4.6 mm, 5 μm) reverse phase column and mobile phase with 0.1% OPA and ACN in the ratio of (50:50 v/v) with a flow rate of 1.0 mL/min. After injecting 50 μL of BKC solution into the HPLC, recorded the spectra, and selected 210 nm for further analysis based on the response of the analyte. The analyte peak was eluted for about 2 min with an asymmetry. In the initial, trial due to silica, columns often exhibited peak tailing and exorbitant retention because of the undesirable ion-exchange interactions between residual silanols on the silica surface and the analytes, accompanied by the high hydrophobicity of cationic surfactants. I tried different ratios of organic solvent (acetonitrile), and a broad peak was eluted very early. Based on the literature, the BKC pK_a is 4.1. Usually, a buffer is the most effective when used within the ±1 pH unit of its pK_a, but it might provide suitable buffering ±2 pH units; from this literature, the buffer changed to sodium acetate pH

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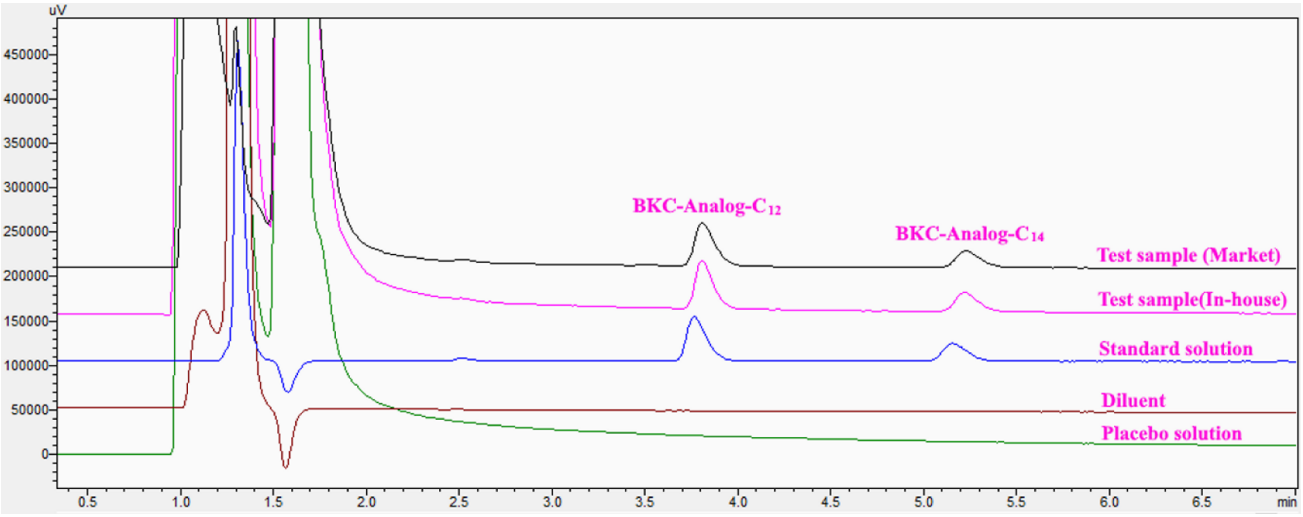

Figure 3. Final chromatogram with the following optimized conditions: a flow rate of 1.0 mL/min, a column temperature of 45 °C, an injection volume of 50 μL, and a wavelength of 210 nm with the Phenomenex Ultracarb C8 (150 × 4.6 mm, 5 μm) column.

5.5 buffer. In the mobile phase prepared with sodium acetate buffer at pH 5.5 and acetonitrile in the ratio of (40:60 v/v), peak shape was not improved and eluted in the shape of "M", which expressed that C_{12} and C_{14} analogs were co-eluted. Organic ratio changes did not help separate the peaks. A pH study was conducted (pH 2.0, 2.5, 3.0, 5.0, and 6.0) and separated the C_{12} and C_{14} analogs. The C_{10} and C_{16} analogs were not detected due to low concentrations. For identifying C_{10} and C_{16} homologs, a high concentration of BKC was injected, which confirmed the separation of four homolog peaks. The elution order was C_{10}, C_{12}, C_{14}, and C_{16}. A suitable resolution was achieved at pH 2.5 buffer with ACN in the ratio of (20:80) v/v with a 1.0 mL/min flow rate. A symmetric peak shape was acquired at a column temperature of 45 °C, and the same was considered throughout the analysis. The final optimized and interference-free chromatogram is shown in Figure 3.

2.4. NaCl Quantification Procedure Optimization. The silver nitrate solution (0.01 M standardized solution) was used to titrate, with potassium chromate solution 5% (w/v) as an indicator. Mohr's method required 1 mL of 0.01 N $AgNO_3$ for 0.5844 mg of the NaCl content. The titration result was re-checked by using an osmometer. Before using the osmometer, the instrument was calibrated using suitable buffer solutions. Ophthalmic solution and nasal spray formulations' individual excipient's osmolality was determined and correlated with theoretical values. The excipient's contents in the formulation can be determined using HPLC methods except for NaCl. Based on those values, a placebo for NaCl (mixture of excipients including API except for NaCl) was prepared. The osmolality values of the placebo, market sample, and in-house sample formulation were checked. After subtracting the placebo osmolality from the sample osmolality, the values with the recovery sample were correlated. The recovery sample was prepared with a known concentration of NaCl solution. Osmolarity results are tabulated in Table S1. Ophthalmic solution 0.1% formulation containing NaCl theoretical osmolality value was 222.2 Osmol/kg. The experimental value obtained from NaCl titration was 211.5 Osmol/kg. The difference between the values was below 5.0%. After placebo subtraction, the in-house and market samples osmolality values were similar to the titration-resulted NaCl osmolality values (209.1 and 208.0 Osmol/kg). This was observed in 0.2% ophthalmic solution and 0.6% nasal spray, which indicates that the NaCl quantity obtained from titration was accurate, and the result was validated by osmolality.

2.5. Specificity. All of the HPLC method's specificity was checked by injecting the individual placebo solutions of each excipient, diluent, and standard solution. All the samples were injected into the DAD system to assess the peak purity. The obtained chromatograms showed peak homogeneity and no interference at the retention time of the analyte peak from the placebo and diluent peaks. These results indicate that the methods are specific. The results are tabulated in Table S2. The UV spectrum and peak purity plots are shown in Figure S2.

2.6. Precision. All the method's repeatability (precision) was evaluated by preparing the 6 individual samples from a homogenous sample and analyzing the samples using the respective methods. The % relative standard deviation (RSD) for six preparations of all the analytes was found below 2.0%, which are 0.9% for EDTA, 0.6% for BKC, 0.9% for DSP, and 1.3% for NaCl. These results conclude that the proposed methods are precise. The results are tabulated in Table S2.

2.7. Recovery. The recovery efficiencies for the analytes DSP, EDTA, BKC, and NaCl were investigated. A known amount of the sample was added to the placebo and quantified by the proposed method at three different levels (80, 100, and 120% of target concertation). The results were found to be between 98.0 and 102.0%, which are in the range of 99.1−99.7% for EDTA, 99.1−99.4% for BKC, 99.8−100.8% for DSP, and 99.7−100.1% for NaCl. The amount added and found was calculated, and the results express the closeness between the measured and true values. The results proved that the methods are accurate for quantifying the compounds. The results are tabulated in Table S2.

2.8. Linearity. EDTA linearity was performed from 2.6 to 15 μg/mL, DSP linearity was performed from 13 to 80 μg/mL, NaCl linearity was performed from 33 to 244 μg/mL, and BKC linearity was performed from 4 to 25 μg/mL. The correlation coefficient values were found to be higher than 0.999, which are 0.9999 for EDTA, 0.9996 for BKC, 0.9992 for DSP, and 0.9990% for NaCl. These results proved that the

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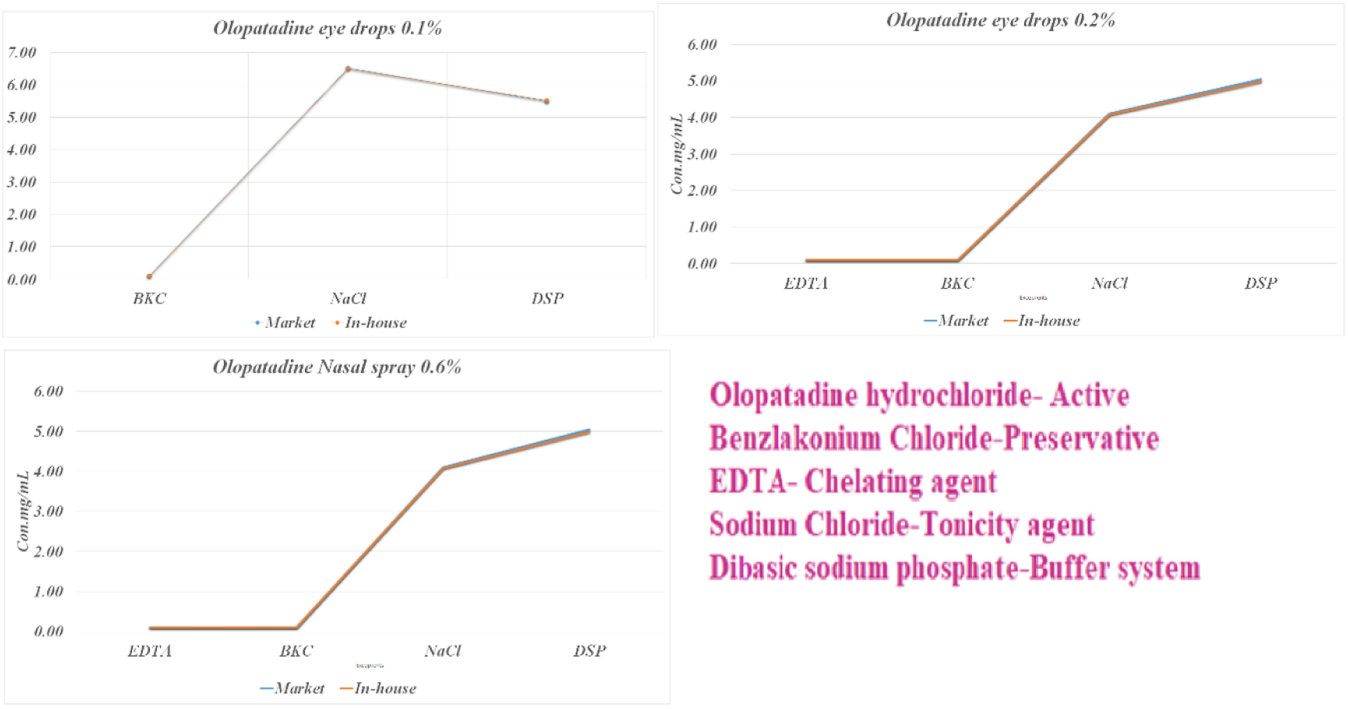

Figure 4. Graphical representation of 3 formulations market and in-house samples excipients quantity sameness.

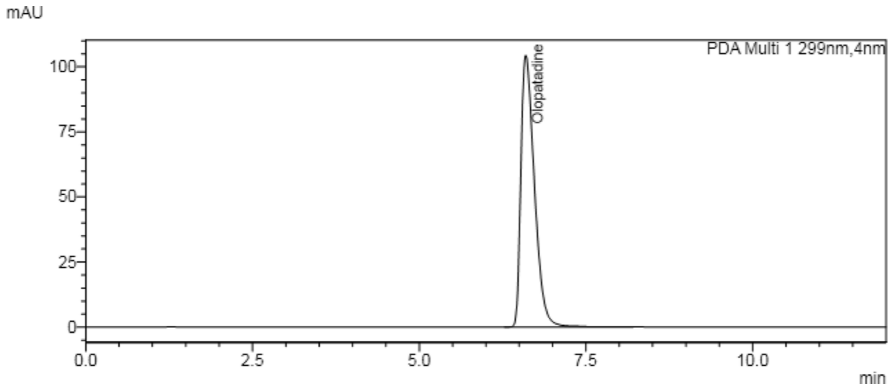

Figure 5. OPT assay determination chromatogram.

method is linear. The results are tabulated in Table S2. The linearity graphs are shown in Figure S3.

2.9. Formulation Sample Evaluation. HPLC and titration methods were used to evaluate the EDTA, DSP, BKC, and NaCl contents of three lots of in-house products and marketed samples. The excipient quantity in the in-house and marketed samples is presented in a graphical representation (Figure 4), expressing the formula's sameness.

The contents of EDTA, DSP, BKC, and NaCl were similar in the in-house manufactured drug product to the marketed product. The physicochemical properties of both products were examined to verify the qualitative similarity of the components. The OPT assay was done using the official USP pharmacopeial method. The sample's viscosity was determined using an Ostwald viscometer. The in-house formulation data matches well with the market sample, and it suggests that the components present in the samples are similar. The results are tabulated in Table S3. Assay determination chromatograms and impurity determination chromatograms is shown are Figures 5 and 6.

3. CONCLUSIONS

Reverse engineering of mast cell stabilizer and histamine receptor antagonist OPT ophthalmic solution 0.1 and 0.2% and nasal spray 0.6% formulations is performed herein. All four methods are specific, and the reported methods have an excellent ability to determine the components in the presence of other ingredients. The protocols can be used for deformulation studies of many other formulation products. The main advantage of this whole process is that it is time-saving and cost-effective due to the shorter run time and simple analytical procedure. The USP ophthalmic solution monograph officially suggests Ultracarb C8 (150 × 4.6 mm, 5 μm). The same column is utilized for EDTA and BKC determinations. It is cost-effective, and as per guidelines, we need to test the EDTA and BKC in the shelf-life analysis. These methods can be utilized in routine quality control and stability sample analyses. All processes are simple and analyst-friendly. By this procedure, OPT ophthalmic and nasal formulation products can be deformulated to prepare generic formulations.

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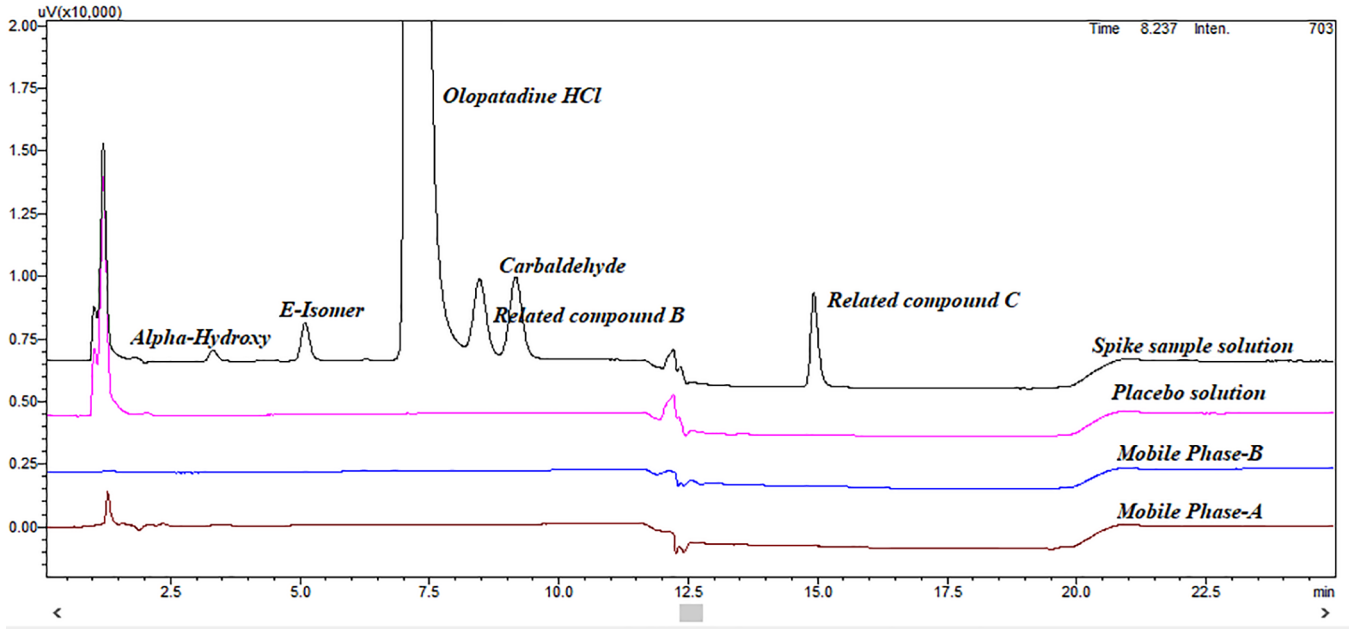

Figure 6. OPT impurity determination chromatogram.

4. MATERIALS AND METHODS

4.1. Chemicals and Reagents. The olopatadine HCl active pharmaceutical ingredient was procured from Chongqing Huabang Shengkai Pharmaceutical Co. Ltd, China. Excipient-grade anhydrous DSP was procured from Chengdu Boon Stream Chemical Industry Co., Ltd, China. Excipient grade BKC (RF-40) was purchased from Hubei Gedian Renfu Pharmaceutical Accessories Co., Ltd, China. Excipient grade EDTA and NaCl were purchased from Merck Ltd, China. Excipient grade hydrochloric acid and sodium hydroxide were procured from Sino Pharm Chemical Reagents, China. Nitric acid, PHT, sodium acetate, orthophosphoric acid, copper acetate, TBAH 10% in water, and glacial acetic acid were procured from Sino Pharm Chemical Reagents, China. The OPT reference standard was procured from NIFD, China. Acetonitrile (ACN) was purchased from Dikma Technology Inc, China. Potassium chromate indicator and silver nitrate were procured for titration from Sino Pharm Chemical Reagents, China. Class "A" glassware and in-house Milli-Q water were used in the research.

4.2. Equipments/Software/Column. Chromatographic columns such as Ultracarb C8 (150 × 4.6) mm, 5 μm column from Phenomenex, and IC-Pak Anion (50 × 4.6) mm, 10 μm from waters were procured for the method development, validation, and regular analysis. A liquid chromatographic machine from Agilent Technologies, model 1100 series, with UV and DAD detectors, was used. The HPLC was operated with Open lab CDS software. Chemicals and salts are weighed on a semi microbalance from Shimadzu with model no AP225WD. Buffer pH 4.5 and 2.5 were prepared using the Mettler pH meter model no FE-28. The buffer was filtered with a vacuum pump from HA diaphragm model no HPD-25B. Precision Systems Touch micro-OSMETTE Osmometer model 6002 was used for osmolality checking.

4.3. Methods. *4.3.1. Determination of DSP.* The mobile phase is prepared by transferring 200 mg of PHT into 100 mL of water mixed and dissolved well and then 5 mL of 100 mM nitric acid was added, mixed well, and diluted to 1000 mL with water. A chromatography system equipped with a UV detector and an IC-Pak Anion (50 × 4.6 mm, 10 μm) HPLC column was used with a 1.0 mL/min flow rate, a 25 °C column temperature, and a 50 μL injection volume. The detection was done at a 288 nm wavelength. The total run time was 8 min. Standard and sample solutions were prepared with water at a 15 μg/mL concentration. HPLC was injected into samples, and the content of DSP present in the sample was calculated. The results are mentioned in Table 1. The placebo solutions are prepared based on the table quantities by excluding DSP.

Table 1. Chemical Compositions of Analyte and Excipients Found in the Marketed Samples are Presented in Percentages[a]

no	name	eye drops 0.2%	eye drops 0.1%	nasal spray 0.6%
1	olopatadine HCl equivalent to olopatadine	0.2	0.1	0.6
2	povidone	0.18	NA	NA
3	BKC	0.01	0.01	0.01
4	DSP	0.5	0.5	0.5
5	NaCl	0.55	0.65	0.41
6	edetate disodium	0.01	NA	0.01

[a]NA: not applicable to the particular formulation.

4.3.2. Determination of EDTA. The mobile phase was prepared by mixing the pH 4.5 buffer (4.1 g of sodium acetate in 1000 mL of water, pH adjusted to 4.5) with a glacial acetic acid solution. 8 mL of 10% TBAH in water was added to pH 4.5 buffer and ACN in the ratio of (90:10, v/v). Diluent was prepared by mixing the pH 4.5 buffer, ACN, and copper II acetate (10 mg/mL) solution in the ratio of (78:20:2, v/v/v). The chromatography system equipped with a UV detector and Ultracarb C8 (150 × 4.6 mm, 5 μm) column was used with a flow rate of 1.0 mL/min, a column temperature of 30 °C, and an injection volume of 50 μL. The detection was done at 260 nm wavelength with a total run time of 10 min. Standard and sample solutions were prepared with diluent at 10 μg/mL

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concentration. For the injected standard and sample solutions, the content of EDTA present in the sample was calculated. The results are mentioned in Table 1. The placebo solutions are prepared based on the table quantities by excluding EDTA.

4.3.3. Determination of BKC. The mobile phase was prepared by mixing the pH 2.5 buffer (4.1 g of sodium acetate in 1000 mL of water, pH adjusted to 2.5 with an orthophosphoric acid solution) and ACN in a ratio of (20:80, v/v). The chromatography system equipped with a UV detector, Ultracarb C8 (150 × 4.6 mm, 5 μm) HPLC, was used with a flow rate of 1.0 mL/min, a column temperature of 45 °C, and an injection volume of 50 μL. The detection was done at 210 nm wavelength with a total run time of 8 min. Standard and sample solutions are prepared with the mobile phase at 15 μg/mL. The sample solutions were injected into the standard and sample solutions, and the content of BKC present in the sample was calculated. Due to the low concentration (product contains only 0.1 mg/mL of BKC), C_{10} and C_{16} analogues were not detected. The results are mentioned in Table 1. The placebo solutions are prepared based on the table quantities by excluding the BKC.

4.3.4. Determination of NaCl. The NaCl determination was carried out using Mohr's titration method. 3.2 mg equivalent NaCl was weighed and transferred into conical flasks containing 25 mL of water. Five drops of potassium chromate indicator were added and titrated with a 0.01 M silver nitrate solution. The endpoint was a brick red color. 1 mL of 0.01 M $AgNO_3$ was used for 0.5844 mg of the NaCl present in the sample, and % of NaCl present in the sample was calculated. The titration results were confirmed by the values obtained using an osmometer. Using suitable calibration buffers, the osmometer was calibrated before analysis. NaCl solutions were prepared between 33 and 244 μg/mL by spiking the NaCl placebo solution. The osmolality was measured, and values were estimated using the linear equation. The osmolality of placebo and sample solutions was measured, the placebo value was subtracted from the sample, the NaCl content was calculated, and the resulting values are correlated with the titration value. The results are mentioned in Table 1. The placebo solutions are prepared based on the table quantities by excluding NaCl.

4.3.5. Quantification of OPT and Its Impurities. Quantification of OPT and impurities was done using the available literature methods. An injection volume of 15 μL and a column compartment temperature of 30 °C were used for a Boston green C8 column (150 × 4.6 mm), 5 μm, and the detection at 299 nm. (75:25) v/v sodium dihydrogen phosphate buffer with pH 3.5 was used as the mobile phase for assay determination.[23] The flow rate is 1.0 mL/min, and the gradient program is time 0.01/0, 13/0, 13.1/90, 18/90, 18.1/0, and 25/0. The column compartment temperature is 30 °C. Mobile phase A consists of 0.1 M sodium dihydrogen phosphate pH 3.5 buffer and 70% acetonitrile solution (70% acetonitrile, 30% organic solution). Anhydrous sodium dihydrogen phosphate pH 3.5 buffer and acetonitrile organic solution in a 50:50 v/v proportion as mobile phase B for impurity determination.[24]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00311.

Osmolality values for individual excipients and OPT compared with theoretical and experimental values for quantifying the NaCl; partial validation data of the analytical methods; market samples and in-house formulations physicochemical properties comparison data; structure of excipients; UV spectrum and peak purity plot for EDTA, DSP, and BKC; and linearity graph for EDTA, NaCl, DSP, and BKC (PDF)

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