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A NOVEL METHOD FOR ANALYSIS OF ACTIVE PHARMACUTICAL INGREDIENTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUES

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Abstract

The titled paper discloses a novel methodology for study and analysis of active pharmaceutical ingredients extracted using the High-Performance Liquid Chromatography techniques. Performance of the proposed novel method is evaluated by targeting the one of the active pharmaceutical ingredients Zipracidone HCl which is used to treat the psychotic disorders like schizophrenia, mania, or bipolar disorder and other similar diseases. The process is carried out through early-stage chromatography preparations, sample solution preparations, linearity preparations, accuracy level preparation and Robustness assessment. The experimental results show 99.73% (as such basis) functional activity of drug and 99.97% (on anhydrous basis) functional activity with average accuracy of 99.57% (as such basis) and 99.88 (on anhydrous basis). Other outcomes are disclosed through the result and discussion section.

Key words: Actie Pharmaceutical Ingredients (API), HPLC, Zipracidone HCl,

Introduction

Active Pharmaceutical Ingredients are the ingredients used in the drugs which helps to cure the diseases and maintain the health of the consumer. Different methodologies and techniques are used for chemicals and pharmaceutical ingredients behaviour and performance analysis as a whole and in combination to the other materials. Based on the requirement different techniques are deployed for analysis of the product like, Titrimetric which is used for assay of bulk drug, spectrophotometric analysis X-ray which is used for structure elucidation purpose, UV spectrophotometry and IR Spectrophotometry which is famous for assay and identification, NMR, AAS, Polarimeter, DSC, TGA, Coulometry, Paper/TLC, GC and various other used for estimation of different elements, study of different molecules, drug excipient interaction, moisture determination, weight loss detection of bulk drug detection, quantification and estimation of volatile solvents and for various other purposes and applications.

In addition to these techniques, High Performance Liquid Chromatography

(HPLC) techniques is also widely used for analysis purposes. We demonstrate use of HPLC techniques for analysis of one of the active pharmaceutical ingredients that is Zipracidone HCl. The Zipracidone HCl is widely used in preparation of the drug which is highly consumed to treat the psychotic disorders like schizophrenia, mania, or bipolar disorder and other similar diseases. The HPLC technique is purposely used to confirm the identity of a drug and provide quantitative results and to monitor the progress of the therapy of a disease. The detailed research review, in-depth experimental set-up, research outcomes and analysis are discussed through the subsequent sections.

Previously Reported Technology

The authors [1] have built up a simple dimension elimination high-performance liquid chromatographic technique for speedy molecular mass screening to identify the average molecular mass and amount of Taizishen polysaccharides extract. The molecular mass calibration graph for polysaccharide standards was found to be linear with a correlation coefficient of 0.99. The proposed screening employed high performance liquid chromatographic column as a mobile phase and refractive index identifier. This technique can be employed to investigate average molecular mass, polydispersity, and quantity of polysaccharides. Furthermore, the screening technique is appropriate for quality control of polysaccharide preparation in medicines and pharmaceutical products.

Ceramides are the most essential signalling particles involved in several cellular procedures that include differentiation, apoptosis and cell development. Recently, several techniques are employed for ceramide investigation, out of which some are cumbersome or insensitive. In this paper, the authors have developed techniques using high-performance liquid chromatography and thin layer chromatography followed by evaporative light spreading identification to identify ceramide directly in cell extracts without derivation which was reproducible and effective. Normal phase high performance liquid chromatography and thin layer chromatography outputs show that yeast includes some kinds of ceramides. [2]

There are many factors influencing the growth and secondary metabolites of callus and saffron callus. For callus cultures, this paper presented the effects of culture conditions, including temperature, light levels, the carbon source, and its concentration. To advance callus development, the greatest way was naphthalene acetic acid and the best possible proportion of naphthalene acetic acid to benzyl amino purine was 0.25:2. It was observed that naphthalene acetic acid advanced the development of saffron callus but had no advantage and may reduce crocin synthesis that had the opposite effect. Gibberellin promoted both development and synthesis. [3]

In the paper [4], a fluid independent ultrasonic technique is presented for flow identification in micro channels in the insensitive atmosphere of a high pressure liquid chromatography system. In the fluid, ultrasonic waves are energized by unconnected media surface acoustic waves. The channel ceiling works as an acoustical mirror for longitudinal ultrasonic waves propagating through the fluid. To reduce the flow of fluid from the ultrasonic communication after reflection, the authors have employed an arrangement of period differential phase and period of flight measurements. To authenticate the practical outputs, the authors have employed an adapted period explicit limited element technique. By varying the propagation track, the authors are capable to reduce the fluid direction over the period with very high efficiency.

The Ellagic acid substance in pomegranate leaf was determined employing high performance liquid chromatography to analyse the effects of different seasons and procedure techniques on the Ellagic acid

level. An investigation of variation shows that the Ellagic acid substance is considerably reliant on the season. It was found that the Ellagic acid substances enhances considerably for the season to the highest level in November to December. The effect of leaf diversity on the Ellagic acid substance is less significant than the season. The procedure techniques have diverse effects on the Ellagic acid substance. The experimental results show that the Ellagic acid substances in different varieties of pomegranate are different in five successive seasons from July to November. [5]

The most generally consumable pharmacologically active content in the world is caffeine, but its frequent consumption may lead to caffeine poisoning. The authors [6] have proposed a technique for the comparative analysis of caffeine consumption in different medicines employing terahertz spectroscopy. Comparative analysis of terahertz spectroscopy with Raman spectroscopy and high-performance liquid chromatography shows that there was less than a 4% difference between terahertz and high-performance liquid chromatography outputs. Moreover, the investigation of caffeine in several medicines was done using the support vector regression technique. The proposed terahertz technique proved that it could attain effective, fast and non-destructive identification of important constraints in medicines.

Worldwide, Thalassemia is considered a widespread hereditary blood syndrome that has gotten excessive deliberation in medical research. Hereditary syndrome has a high risk that a child will get these syndromes from his guardians. In the study, it was found that, if both guardians have Thalassemia, then there is a 30% chance that the child will be a Thalassemia carrier. An appropriate way to handle Thalassemia is prenatal analysis. In this paper [7], the identification of Thalassemia is done by estimating red blood cell indices from the whole blood count test. Three machine learning algorithms are employed named Random Forest, support vector machine and gradient boosting machine.

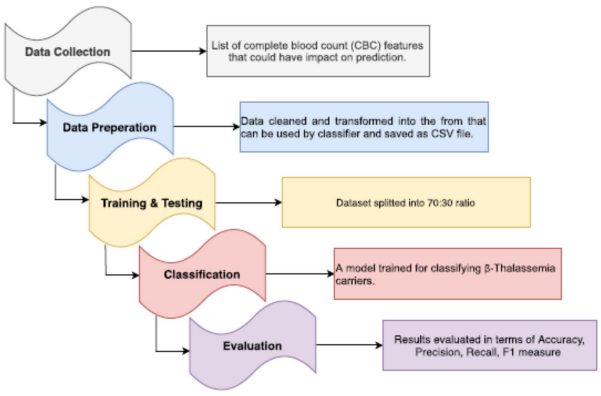

Fig. 1. Phases of Methodology Steps

In the paper [8], the cytotoxicity and apoptotic effect of phytoceramide was analysed by utilizing methoxy polyethylene glycol cholesteryl succinate and cholesteryl hemisuccinate. The ceramide containing liposomes prepared to utilize ultrasonication contained cholesteryl hemisuccinate and phytoceramide. The experimental outputs show that the ceramide entrapment efficiency is 92% and the proportion of ceramide to cholesteryl hemisuccinate affects the liposome characteristics. The phytoceramide encapsulated in the liposome decreases the

cellular activity of the murine mastocytoma cell in dose dependent manner and the reduction of cellular activity.

To examine the nacre environment proteins and their effects on the calcium carbonate crystal, a soluble environment protein with a perceptible molecular mass was isolated from fragmented nacre of pearl oysters treated with sodium hydroxide solution. The protein was defined by reversed phase high performance liquid chromatography and gel segregation chromatography. R14 can introduce stimulated calcium carbonate crystal development and accelerate aragonite precipitation. The proposed investigation recommended that two proteins are associated with the inexplicable organic matrix by disulfide bridges because the extraction yield increases when mercaptoethanol is mixed with the medium. [9]

All over the world, uncontaminated drinking water is becoming one of the most important benefits of the rising population. The most important threat to the accessibility of uncontaminated drinking water is given by agricultural and industrial sectors leads to water contamination. The authors [10] have proposed a new, simple to manufacture and flexible amperometric sensor comprised of silver-coated electrodes with a copper film deposited on top over a carbon annotate. The copper electrode provides exceptional catalytic movement towards the decrease of nitrate ions at the neutral potential of hydrogen (pH) with a considerable growth in cathode peak currents. In the investigation, it was found that the sensor shows excellent conformity with the compared result of high-performance liquid chromatography measurements, proving to be a capable investigating tool for the identification of nitrate in water.

Development and Validation of the Material

Development and validation of the Zipracidone HCl is carried out using the Assay Method. The materials and the solutions which are used in the process are disclosed through the following table.

Table 1: Materials and Solutions

Sr. No.	Name of the Chemical	Grade	Manufacturer
1	Acetonitrile	HPLC	MERCK
2	Methanol	HPLC	MERCK
3	Triethylamine	AR	Qualigens
4	Ortophosphric acid	AR	Qualigens
5	Milli Q Water	HPLC	Millipore

The chromatographic conditions involve the parameters as disclosed in the subsequent table.

Table 2: Components and Particulars

Sr. No.	Items	Particulars
1.	System	Systronic HPLC with Empower Software
2.	Column	YMC pack C18, 250 x 4.6 mm, 5µm
3.	Flow Rate	1.0 ml/minute
4.	Wavelength	230 nm
5.	Injection Volume	10 µl
6.	Column Temperature	30°C
7.	Diluent	Mobile phase as diluents.
8.	Run Time	30 min

The buffer solution is prepared by taking 3.0 ml of triethylamine and adjusting the PH to 3 with orthophosphoric acid. The mobile phase is prepared by taking the 550 ml of buffer, 160 ml acetonitrile and 320 ml of methanol. The Zipracidone working standard solution is prepared by taking 50mg of Zipracidone acid transfer into 50ml volumetric flask dissolve and diluted up to the mark with diluent. The sample solution of Zipracidone is prepared by taking 50 mg of Zipracidone sample transferring in to 50 ml volumetric flask. Dissolved and diluted up to the mark with diluent. The percentage of assay is calculated using the following formula:

$$\% \, Assay = \frac{AT * WS * P * 100}{AS * WT * (100 - Water\ Content)}$$

The linearity of the proposed method was learned by injecting the solutions prepared at different concentration levels is prepared by taking 500 mg of Zipracidone into 55 ml volumetric flask, dissolved and diluted up to the mark with diluent at 10000 ppm.

- 5 ml of Zipracidone working standard solution is considered in 50 ml volumetric flask and diluted up to the mark with diluent at 1000 ppm.
- 8 ml of Zipracidone working standard solution is considered in 50 ml volumetric flask and diluted up to the mark with diluent at 1500 ppm.
- 10 ml of Zipracidone working standard solution is considered in 50 ml volumetric flask and diluted up to the mark with diluent at 2000 ppm.
- 12 ml of Zipracidone working standard solution is considered in 50 ml volumetric flask and diluted up to the mark with diluent at 2500 ppm.
- 14 ml of Zipracidone working standard solution is considered in 50 ml volumetric flask and diluted up to the mark with diluent at 3000 ppm.

Accuracy of the proposed methodology was studied by injecting sample solutions prepared at three different concentration levels in presence of fixed concentration of impurities as given below. For preparation of the standard working solution Zipracidone weighed accurately 50 mg transferred into 50 ml volumetric flask, dissolved, and diluted up to the mark with diluent. Pipette out 5 ml above solution in 50 ml volumetric flask diluted up to mark with diluents. Out of three samples, the first sample is prepared by taking the 50.20 mg, 50.22 mg and 50.24 mg of Zipracidone material in three different 50ml volumetric flask. Subsequently, 10 ml of diluents are dissolved and 2.5 ml of standard stock, prepared earlier, is added, dissolved, and diluted. The second sample was prepared by taking 50.30 mg, 50.40 mg, and 50.60 mg of Zipracidone material in three different 50 ml volumetric flask followed by 10 ml diluents dissolved and 5.0 ml of std stock was added dissolved and diluted. Finally, the third sample was prepared by taking 50.18 mg, 50.28 mg and 50.62 mg of Zipracidone material in three different 50 ml volumetric flask and 10 ml diluent dissolved and 7.5 ml of standard stock was added, dissolved and diluted.

Robustness of the method was verified by purposely changing the parameters like flow rate, pH of buffer solution and mobile phase composition. 60 mg of Zipracidone material is sampled and transferred into the flask, dissolved, and diluted up to the mark with diluent. 8 sample solutions of zipracidone samples are prepared in the same fashion. Diluent, Zipracidone working standard solution in eight replicate injections and eight sample solutions of Zipracidone are injected into the HPLC chromatograph. Relative standard deviation of peak areas for eight replicate injections of Zipracidone working standard solution calculated. Assay of eight samples of

Zipracidone and relative standard deviation of assay values are calculated.

Performance Evaluation

Accordingly, performance evaluation of the assay method is recorded and disclosed through the following table.

Table 3: Precision Evaluation of Zipracidone HCl

Sr. No.	Sample Number	Weight of Sample (mg)	Area	% Assay (As Such Basis)	% Assay (On Anhydrous Basis)
1.	1	50.18	2859889	99.66	99.97
2.	2	50.20	2889480	99.65	99.95
3.	3	50.22	2874648	99.65	99.98
4.	4	50.24	2867445	99.67	99.96
5.	5	50.28	2874825	99.68	99.94
6.	6	50.30	2874322	99.67	99.95
7.	7	50.40	2880530	99.64	99.97
8.	8	50.60	2870540	99.65	100.00
AVG		50.30	2873960	99.65875	99.965

The subsequent table discloses the findings of linearity calculations, disclosed through the table 4

Table 4: Linearity Determination of Zipracidone HCl

No.	Level-1 (1000 ppm) (Area)	Level-2 (1000 ppm) (Area)	Level-3 (1000 ppm) (Area)	Level-4 (1000 ppm) (Area)	Level-5 (1000 ppm) (Area)
1.	1438685	2297580	2877589	3453107	4308166
2.	1442457	2296445	2866856	3454189	4308655
3.	1436798	2297480	2871856	3454567	4308783
AVG	1439313	2297168	2872100	3453954	4308535
				Correlation Coefficient (r)	0.9999

The subsequent figure 1 depicts the linearity graph recorded after execution of the proposed method.

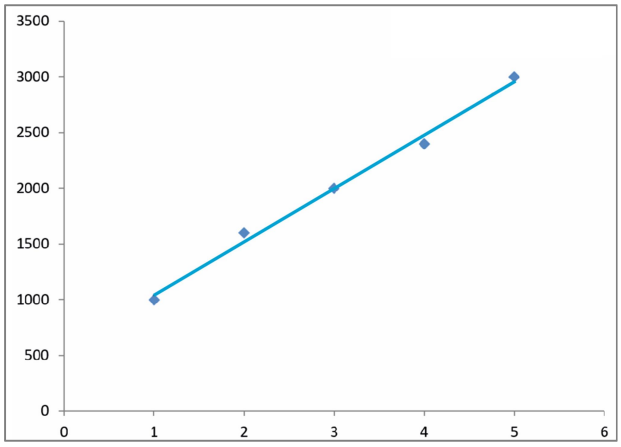

Fig. 1: Linearity Graph of the Zipracidone HCl

The subsequent table 5, 6, and 7 discloses the different levels of accuracy observed for distinct samples .

Table 5: Accuracy Level-1 of Zipracidone HCl

Sample No.	Weight of Sample (mg)	Area	(%) Assay (As Such Basis)	(%) Assay (On Anhydrous Basis)
1	50.19	2296895	99.34	99.82
2	50.20	2297645	99.88	100.00
3	50.25	2296897	99.51	99.81

Table 6: Accuracy Level-2 of Zipracidone HCl

Sample No.	Weight of Sample (mg)	Area	(%) Assay (As Such Basis)	(%) Assay (On Anhydrous Basis)
1	50.51	2877809	99.41	99.69
2	50.42	2877590	99.52	99.41
3	50.59	2877649	99.51	99.69

Table 7: Accuracy Level-3 of Zipracidone HCl

Sample No.	Weight of Sample (mg)	Area	(%) Assay (As Such Basis)	(%) Assay (On Anhydrous Basis)
1	50.19	3453269	99.59	99.99
2	50.21	3453450	99.71	99.94
3	50.39	3453501	99.61	99.91

Subsequently the robustness of the method is also calculated for the flow rate of 0.9 ml/min and 1.1 ml/min, accordingly the outcomes are disclosed through the following table 8 and table 9.

Table 8: Robustness for Flow Rate of 0.9ml/min

Sample No.	Weight of Sample (mg)	Area	(%) Assay (As Such Basis)	(%) Assay (On Anhydrous Basis)
1	50.10	2657810	99.49	99.79
2	50.20	2657250	99.59	99.94
3	50.31	2657199	99.61	99.82

Table 9: Robustness for Flow Rate of 1.1ml/min

Sample No.	Weight of Sample (mg)	Area	(%) Assay (As Such Basis)	(%) Assay (On Anhydrous Basis)
1	50.07	3077780	99.62	99.93
2	50.14	3077580	99.67	99.98

3	50.16	3077537	99.64	99.95

Further the robustness is also calculated for the different pH plus possibilities. The outcomes are disclosed through the following table 10 and table 11.

Table 10: Robustness for pH Plus = 2.8

Sample No.	Weight of Sample (mg)	Area	(%) Assay (As Such Basis)	(%) Assay (On Anhydrous Basis)
1	50.05	2956994	99.60	99.91
2	50.07	2956895	99.68	99.99
3	50.12	2957111	99.58	99.89

Table 9: Robustness for pH Plus = 3.2

Sample No.	Weight of Sample (mg)	Area	(%) Assay (As Such Basis)	(%) Assay (On Anhydrous Basis)
1	50.20	2757512	99.58	99.89
2	50.22	2757522	99.55	99.86
3	50.36	2757650	99.65	99.96

The chromatogram of the Zipracidone HCl recorded under different circumstances are disclosed through the following figures.

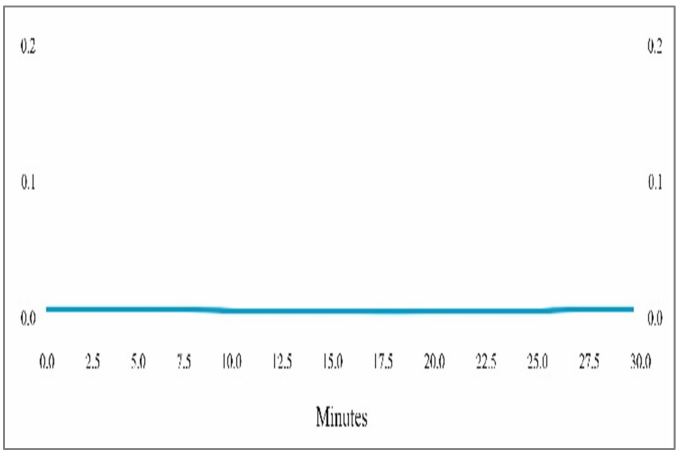

Fig. 2: Chromatogram of the Zipracidone HCl Blank

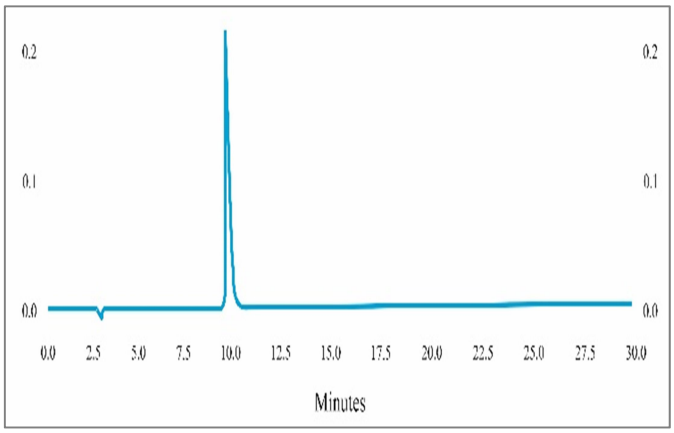

Fig. 3: Chromatogram of the Zipracidone HCl Standard

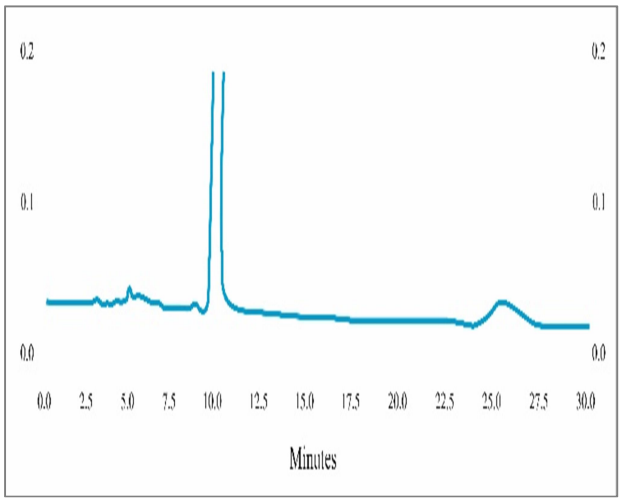

Fig. 4: Chromatogram of the Zipracidone HCl Alkali Degradation

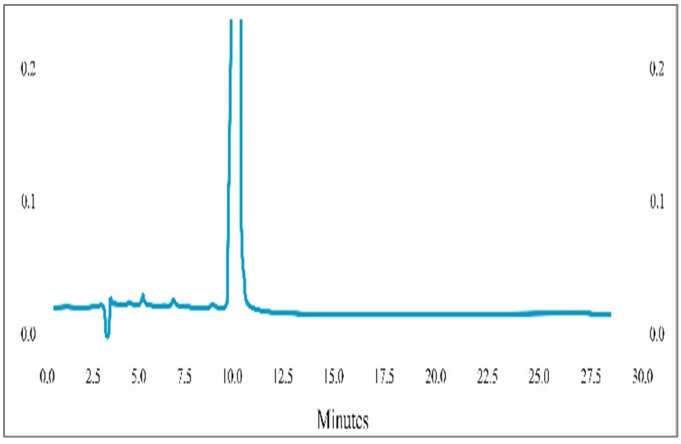

Fig. 5: Chromatogram of the Zipracidone HCl Acid Degradation

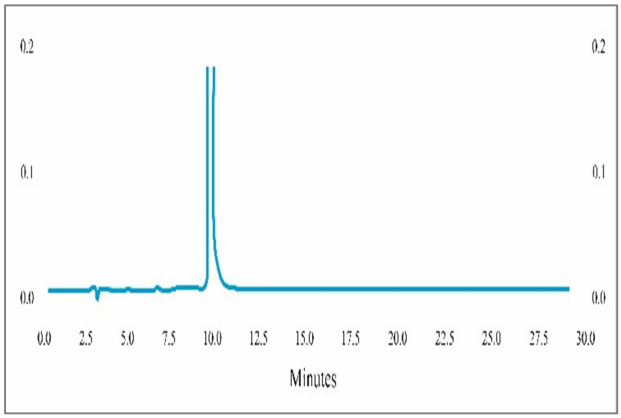

Fig. 6: Chromatogram of the Zipracidone HCl Peroxide Degradation

Conclusion

A novel methodology for the study and analysis of the active pharmaceutical ingredients which are extracted using the HPLC technique is proposed and targeted on the one of the important ingredients, which is used to treat the different psychotic disorders, that is Zipracidone HCl. The process is carried out through early-stage chromatography preparations, sample solution preparations, linearity preparations, accuracy level preparation and Robustness assessment. The experimental results show 99.73% (as such basis) functional activity of drug and 99.97% (on anhydrous basis) functional activity with average accuracy of 99.57% (as such basis) and 99.88 (on anhydrous basis). Under different stress conditions with 1.0 N HCl, 0.1 N NaOH and 5% H_2O_2 kept at room temperature for 30 minutes, UV exposure for 90 hours, thermal degradation at 60^0 C for 10 days and 100^0 C for 24 hours and finally, reflux degradation with water at 90^0C, indicated lowest degradation of 0.06% with the peak purity of 0.96 in 99.80% area of the Zipracidone HCl under thermal degradation at 60^0C and highest degradation of 22.97% is observed with 0.96 peak purity 76.89% area of the Zipracidone HCl under 1.0 N HCl.

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